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THE
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A BI-MONTHLY JOURNAL

**Devoted to the Advancement and Correlation
of the Biological Sciences**

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EDITORIAL

With this, its 85th volume, THE AMERICAN NATURALIST becomes the journal of the American Society of Naturalists. Thus at a ripe age in the life of each party there is consummated a union between a periodical and a society which had long shared both name and interests yet had no formal connection.

THE AMERICAN NATURALIST* which began publication in 1867 had been edited and published for many years (1907–1939) by Dr. J. McKeen Cattell, in association with his son, Mr. Jaques Cattell, and since 1943 (except for a brief interlude) by Mr. Jaques Cattell. It was he who in 1941 sought the collaboration of the American Society of Naturalists which appointed a rotating board of consulting editors, while Mr. Cattell remained as editor and publisher. During 1950 the Society, at Mr. Cattell's request, designated an editor, and after polling its members decided by a very large majority to adopt THE AMERICAN NATURALIST as its journal. The agreement which was ratified unanimously by a membership meeting held in Columbus Ohio on September 12, 1950 gives the Society complete editorial control and provides a subscription to THE AMERICAN NATURALIST for each member of the Society in good standing. In effect, questions of content will be decided by the Society through its editor and editorial board; questions affecting the form and frequency of publication and all business matters will be decided by the publisher; but major questions of any kind will be decided only after consultation between the editors and the publisher.

The American Society of Naturalists was organized in 1883. Its constitution states that its object shall be "the association of working naturalists for the discussion, advancement and diffusion of knowledge concerning the broader biological problems, including organic evolution, thus serving to correlate the various biological sciences into a common philosophy of biology." Its function "as a general rather than a specific society" was confirmed in 1931 by the adoption of a report on policy which has governed the Society's activities until this year. Now to its usual functions of arranging annually a symposium, and an address by its president is added the editing of a journal.

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*The history of THE AMERICAN NATURALIST has been described by Professor E. G. Conklin in the issue of January, 1944 (Volume 74, pp. 29–37). This issue contains also two other papers prepared for the 75th anniversary of the journal by L. C. Dunn (The Naturalist in America) and Paul B. Sears (The Future of the Naturalist).

The future character of THE AMERICAN NATURALIST will be determined by three factors: the objectives of the Society, the past history or traditions of THE AMERICAN NATURALIST, and the changing needs of biology and biologists. The first two have been in general conformity for most of the life of the Society, that is to say, the journal actually did what the Society would have had it do. The reason for this parallelism is obvious: both were responses to the great surge of interest in natural history and in general biology for which the motive force and the unifying idea were provided by the theory of organic evolution. During the first half of the existence of THE AMERICAN NATURALIST and the early years of the Society, the center of interest was in taxonomy, description, exploration, field and museum work, and in methods in support of these; whereas in later years, as genetics became increasingly useful in the experimental study of the factors of evolution, this field came to occupy an increasing amount of space in the journal and of interest in the Society. Likewise a prominent characteristic of the journal came to be its publication of general addresses and of papers presented at symposia of various biological societies including those sponsored by the Society as its chief activity. Thirdly, THE AMERICAN NATURALIST for many years provided a medium for the publication of short reports and discussion of new biological research of the same kinds as those which interested the members of the Society.

Current correspondence with biologists indicates that these are all considered to be useful functions of the journal, of which none except the publication of research reports in evolution and in genetics is adequately served by other journals. In addition it appears that one function, only partly served by THE AMERICAN NATURALIST in the past, is felt as a distinct need by many biologists. The one word which serves as the common denominator for the various forms in which this need has been expressed is synthesis. The kind of synthesis which biologists want is that which arises out of a really adequate analysis both theoretical and factual, and this is most likely to occur in connection with a specific problem to which someone has devoted, with the enthusiasm which accompanies discovery, both the thought and the labor of actual investigation. Biologists probably intend the same meaning when they say they want to see the problem in its larger context, in the perspective of some general biological view.

We may say that THE AMERICAN NATURALIST should evolve in these three directions: (1) To provide a medium for the publication of general addresses and essays and the symposia of biological societies which in the opinion of the editorial board contribute substantially to the main purpose of the Society in "correlating the various biological sciences"; (2) To publish quickly brief reports of new research of general biological interest and brief comments and discussion of these, including comments and criticism of material published elsewhere; (3) To publish papers in which the chief interest inheres in the theoretical interpretation and the synthesis referred to above.

To say this however is to express a hope rather than to create this kind of journal by fiat, for what is to be published must obviously be a reflection of what biologists will provide. Certainly until the membership of the editorial board is completed and has some experience, no radical or permanent changes need be envisaged.

In the meantime the nucleus of the editorial board will be guided by the views of members which have been expressed in meetings and correspondence. THE AMERICAN NATURALIST may be expected to move away from the publication of extensive data papers for which adequate outlets already exist; to become more hospitable to criticism, comment and brief reports especially when these can be put in the form of letters to the editor, and to increase its invitations to naturalists to review briefly new advances in their fields. The editors will be glad to receive manuscripts of these kinds and, for the present, of the kinds which the NATURALIST has published in the past, on any field of biology or natural history. In these ways, it hopes to serve not only members of the American Society of Naturalists but biologists in all countries and has no intention of favoring either members or Americans in choosing material for publication.

THE EDITORS

To Members of the American Society of Naturalists:

This issue is being sent to all members of record whether or not they have paid membership dues for 1951. Subsequent issues can be sent only to paid-up members. Members who have not yet done so will please remit dues ($5.00 including subscription) to:

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INTERBREEDING OF NATURAL POPULATIONS OF VERTEBRATES[1]

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INTRODUCTION

The rate of divergence of natural populations of both plants and animals is dependent on the amount of gene flow between the populations. Reduced gene flow obviously favors differentiation, while free gene exchange tends to prevent divergence of populations. The interbreeding, or hybridization, of different populations in nature permits gene exchange between such populations, and the amount of gene transfer is dependent on the amount of hybridization. Introgressive hybridization of subspecies, species, and genera has been given much weight as a major cause of the variability in existing populations of plants by Anderson and Hubricht (1938), Anderson (1948, 1949), Heiser (1949) and others of this same group. For purposes of comparison, it seems profitable to discuss the occurrence of, the apparent basis for, and the evolutionary implications of hybridization in nature between populations of mammals and between populations in other vertebrate groups. No attempt has been made, however, to review all of the widely scattered literature on hybridization of vertebrates. Most of my examples will be drawn from a few groups of vertebrates, including Peromyscus among the mammals, the acaudate amphibians, and certain families of fishes.

The stage of speciation of two populations will presumably affect their potential rate of gene exchange. The probable course of speciation will be reviewed, therefore, as a background for discussing the interbreeding of natural populations. We believe that geographic isolation of populations is usually the initial step in speciation (see also Mayr, 1942, 1947). Once geographic isolation occurs, the populations are free to diverge in many ways. They may come to differ morphologically, physiologically, and psychologically, and their hereditary materials may eventually become so different that the two populations would no longer produce hybrids. They may come to differ in habitat preference, in place of breeding, and in their response to various environmental factors associated with the initiation of reproduction. If any combination of these characters will act to prevent interbreeding, that is, as isolating mechanisms, between the two populations, these populations can come to occupy the same range without losing their distinctness through interbreeding. Most modern biologists would define such reproductively isolated populations as species. Because evolution is a continuing process, we can expect at any moment in time to find natural populations in all stages of speciation.

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[1] Read at a symposium on "The Role of Introgression in Evolution," before The Society for the Study of Evolution, Columbus, Ohio, September 11, 1950.

If the species is defined on the basis of reproductive isolation, there should be a fairly sharp cleavage between cases of the interbreeding of species and the interbreeding of populations that have not reached this stage of evolution. In the case of species, hybridization involves the breakdown of the mechanisms of reproductive isolation. The fact that isolating mechanisms do break down under some circumstances has necessitated a revision of the biological definition of species to admit the possibility of some gene exchange (see Mainland, 1942; Mayr, 1949). In the case of intraspecific populations, interbreeding occurs between populations that have not developed effective isolating mechanisms. Our position on the classification of natural hybrids is that sympatric species have demonstrated their stage of speciation by their co-existence in nature (see also Mayr, 1942). Hybrids between such populations would be regarded unquestionably as interspecific hybrids, resulting from the breakdown of the isolating mechanisms that act to maintain the distinctness of the two populations. Classification of geographically separate populations is difficult, because the stage of speciation is unknown in the case of most of these populations. The conventional taxonomic practice is to classify such isolated populations as species if there is morphological differentiation. This practice accounts for much of the looseness of usage of the term interspecific hybrid in the current literature.

A classical example of the weakness of morphological criteria in evaluating isolated populations is given by Clausen, Keck, and Hiesey (1947). A new genus on morphological grounds proved, on breeding experiment, to be a subspecific population of a previously described species, *Layia glandulosa*. Mayr's (1948) suggestion that all isolated, allopatric populations be called subspecies until proved otherwise has some merit, but Mayr agrees that, "it is just as serious an error to call a population a species if it is really only a subspecies than to call it a subspecies if it is a species." Whatever expedient we use for purposes of classification of such isolated populations, we should keep in mind that such populations may or may not have acquired intrinsic isolating mechanisms. Previously isolated populations that have extended their range to interbreed in a zone of contact are easier to classify. The interbreeding of these populations in the zone of contact is evidence that these populations were in an incipient stage of speciation. We would regard hybrids between such populations as intraspecific, not interspecific hybrids.

In the following discussion, we will treat separately cases of interspecific hybridization and cases of interbreeding between the various categories of intraspecific populations.

HYBRIDIZATION OF SYMPATRIC SPECIES

In discussing hybridization of sympatric species, we are interested in whether or not such species can hybridize, in whether or not they do hybridize in nature, in the frequency with which hybrids are produced, and in the evolutionary significance of such hybridization.

Fertility Between Species

The potentialities for natural hybridization of species have been shown to exist in all vertebrate groups that have been adequately studied. It has become increasingly evident in recent years that many closely related species are at least partially interfertile. Members of the same species group in Peromyscus can be crossed in the laboratory to produce at least some fertile F_1 hybrids when no choice of mates is given (see Dice, 1933, 1937; Dice and Liebe, 1937; Watson, 1942; W. F. Blair, 1943a). There is similar evidence from widely scattered groups of vertebrates, including: toads and tree-frogs (A. P. Blair, 1941b); ranid frogs (Moore, 1946); cyprinodont fishes (R. R. Miller, 1948). Numerous cases of interspecific hybrids in birds are summarized by Mayr (1942). More interspecific hybrids have been produced in the laboratory in Drosophila than in any other group of animals. Patterson (1942) listed thirty-one interspecific hybrid combinations, and he informs me that the number now has more than doubled. It seems to be generally true that diverging populations of animals usually acquire mechanisms of reproductive isolation before they have diverged genetically to the point of intersterility.

So-called intergeneric hybrids are not distinguishable from interspecific hybrids. We are dealing in both cases with hybrids between representatives of different populations of animals. Genera are subjective units of classification. Most intergeneric hybrids are described from fishes and birds, the groups of vertebrates in which there has been the greatest amount of taxonomic splitting.

Occurrence of Sympatric Hybrids

Because closely related species are frequently interfertile, the probability of species populations hybridizing in nature depends largely on the effectiveness of the isolating mechanisms that prevent fertilization of the eggs of one species by the sperm from another, related species. Because the mode and pattern of reproduction vary greatly within the vertebrates, it is not surprising that different complexes of isolating mechanisms have been developed in different groups. It is to be expected that some groups have developed more effective complexes than have others. Present information about complexes of isolating mechanisms in several groups of vertebrates has been summarized in table 1. Some significant conclusions can be drawn from this comparison. The most important is probably that in every case studied there are several mechanisms involved in keeping the species separate. The combination of mechanisms varies from group to group, but complexity of factors is characteristic of all groups. The complex of factors appears more susceptible to breakdown in some groups than in others.

TABLE 1

COMPLEXES OF ISOLATING MECHANISMS REPORTED FROM SEVERAL GROUPS OF VERTEBRATES

Group	Mode of reproduction	Isolating mechanisms							Authority
		Habitat preference	Breeding season	Call	Mating preference	Courtship pattern	Sexual dimorphism	Sterility	
Bufo (amphibia)	Ext. fertilization; breeding swarms.	Partial	Partial	Yes	No	No	Slight	Slight or none	A. P. Blair (1941a)
Hylidae (amphibia)	”	”	”	”	”	”	”	”	A. P. Blair (1941b)
Peromyscus (mammals)	Int. fertilization; permanent pairs.	”	No	No	Yes	Unknown	No	Slight to none	Papers by Dice, W. F. Blair, Blair and Howard
Centrarchidae (fishes)	Ext. fertilization; nest in colonies.	”	Little or none	”	Probably	Yes	Some	Yes	Hubbs and Hubbs (1932), Bailey and Lagler (1938)
Cichlidae (fishes)	Ext. fertilization; pair formation and care of progeny.	”	?	”	Postulated	?	?	”	Kosswig (1947) Trewavas (1947)
Poeciliidae (fishes)	Int. fertilization.	Slight	?	”	Yes	Yes	Yes	?	Haskins and Haskins (1949)

Groups that practice external fertilization and form breeding swarms are more apt to experience breakdown of isolating mechanisms than are groups that practice internal fertilization and pair during reproduction. Hubbs and Hubbs (1932) believe that interspecific hybrids in the Cyprinidae are, "due to the chance meeting of egg and sperm of distinct species." Groups in which there is brief pairing at the time of fertilization are more susceptible to mis-mating than are groups in which there is more or less permanent pairing and care of the progeny. As Mayr (1942, p. 262) puts it, in the latter case the animals have not only committed an original mistake but have apparently not corrected it afterward. The occurrence of natural interspecific hybrids has been reported in several groups of vertebrates, and these will be discussed below in relation to the isolating mechanisms that pertain in these groups.

The toads (Bufo) are representative of animals that form breeding swarms. Apparent hybrids have been described by A. P. Blair (1941a) between sympatric populations of toads classically treated as species and so considered by him. The principal mechanisms that prevent interbreeding between species of toads are: (1) partially different times of breeding, (2) partially different places of breeding, (3) different calls, although the effectiveness of call differences has not been demonstrated experimentally. There is considerable evidence that, once individuals are in close proximity, mating preference is of little importance as an isolating mechanism. Sexually excited males will clasp objects of appropriate size, even inanimate ones. When two species are breeding in the same pond, a female going to the call of a male of her own species might approach and be clasped by a male of another species. W. A. Thornton (unpublished data) has found cross-mated *Bufo woodhousii* and *Bufo valliceps* near Austin, Texas.

Fishes, with their wide range of breeding habits and consequent variation in complexes of isolating mechanisms, vary greatly in their potential susceptibility to interspecific crossing. Breeding swarms of pelagic spawners are presumably highly specific in their response to external stimuli, discharging eggs and sperm in response to these stimuli (see Mayr, 1942). Cross-fertilization could result from the chance mixing of gametes from different breeding swarms. The family Centrarchidae, a group in which natural hybridization had been widely studied (Hubbs and Hubbs, 1932; Bailey and Lagler, 1938; and others), appears to be rather susceptible to the breakdown of isolating mechanisms when the environment is disturbed by man. These fishes, "have a complex mating behavior, involving a nuptial gyration over the redd ('nest') prepared by the male" (Hubbs and Hubbs, 1932). However, "many females may visit one nest, and one female may visit many nests" (Breder, 1936). This last increases the chance for a mistake, where more than one species is spawning in the same locality. Noble (1934) and Breder (1936) have shown that in some species, at least, males will court inanimate objects irrespective of their general appearance when they are manipulated somewhat to resemble the actions of a female ready to spawn. This tends to indicate that differences in appearance of the females of different species may be of little importance in identifying the females to the males. The final decision apparently rests with the females (see Breder, *op. cit.*), and it seems likely that coloration of the males may affect the decision of a female to approach or not approach the nest occupied by a male. Breder (1936) listed several factors that he considered favorable to the hybridization of species of centrarchids; these include: "the species are numerous; there is less geographic separation than usual; spawning occurs at about the same temperature threshold; spawning sites are limited and similar for most species; nests are exchanged among species." These observations by Breder would seem to indicate that the principal mechanism that prevents cross-fertilization in the sunfishes is the courtship pattern. Environmental factors, however, are indicated by others to be of considerable importance. Bailey and Lagler (1938) found all possible hybrid combinations between three sympatric species in an artificial lake where gravel bottom for nesting was scarce and in which all species were stunted due to overcrowding. Crowding of the populations and reduction of available spawning sites seem to be the chief factors responsible for hybridization in this case. Hubbs and Hubbs (1932) also mention the association between reduced nesting areas and species hybridization in small lakes. Hubbs and Hubbs (1933) and Bailey and Lagler (1938) believe that the sunfish hybrids are sterile. If this is the case, it is hybrid sterility that prevents gene transfer between the species populations. The other isolating mechanisms serve principally to reduce the wastage of gametes in the production of sterile hybrids.

Isolating mechanisms in a group of fishes that practices internal fertilization have been recently studied by Haskins and Haskins (1949). Species discrimination and mating preference were demonstrated experimentally in three sympatric species of the family Poeciliidae. Their evidence indicates tentatively that discrimination rests with the male, although, "marked sexual dimorphism exists on the part of the male in form, coloration, and courtship pattern."

Permanent pairing, at least during the breeding season, and care of the young by both sexes is common among African species of the family Cichlidae (Kosswig, 1947). This is a reproductive pattern that should operate strongly against interbreeding of species populations, and Kosswig believes that it has, "been able to play an important part in the acceleration of species formation *in loco* in the Cichlidae of the great East African lakes."

Mice of the genus Peromyscus show patterns of reproductive behavior and complexes of isolating mechanisms that may be of widespread occurrence in the mammals. In the two species of Peromyscus that have been studied, the mice form more or less permanent pairs that generally exist so long as both mice remain alive (Howard, 1949; W. F. Blair, 1951). Care of the young by the male as well as by the female has been reported from this genus (Horner, 1947) and from a related genus, Baiomys, (W. F. Blair, 1944a). Mating preference, shown experimentally between *Peromyscus maniculatus* and *Peromyscus polionotus* by Blair and Howard (1944), is probably an isolating mechanism of major importance in this group. Ecological isolation also appears to be important.

Only one interspecific hybrid combination has been reported from nature in Peromyscus. Four presumed hybrids between *Peromyscus leucopus* and *Peromyscus gossypinus* were reported from Alabama by Howell (1921). These species cross fairly readily in the laboratory and produce fertile F_1 hybrids of both sexes. The two populations are partially separated in nature by the preference of leucopus for higher, drier forests and the preference of gossypinus for lower, moister forests. Two other sympatric species, truei and nasutus, have been crossed in the laboratory to produce fertile female and sterile male hybrids, but no recognizable hybrid between these species has been found in nature. Mating preference seems to be a strong isolating mechanism in both of these cases.

Birds are like mammals in that the young are cared for by one or both parents and in that permanent pairs are formed in many groups during the breeding season. Mayr (1942) points out that, "sympatric hybrids are formed primarily in genera in which copulation is not preceded by pair formation and an 'engagement period.'" "Hybrids occur much more rarely among pair-forming species of birds."

Chapin (1948) has recently reported presumed hybrids involving three sympatric species of paradise flycatchers in Africa due to loss of ecological isolation following clearing of the rain forest. If confirmed, this will be a remarkable case among the higher vertebrates, for these birds form pairs and both parents care for the young. The apparent dependence of these three populations on ecological barriers as isolating mechanisms strongly suggests that these populations are in a much less advanced stage of speciation than their morphological differentiation would seem to indicate.

Why Are Species Hybrids Scarce?

Although natural hybrids between sympatric species have been reported from many vertebrate groups, the discovery of such hybrids is a comparatively rare event. Hybrids between sympatric species do appear to be much more rare in animals than in plants. Mayr (1942) reported that only two or three dozen hybrids are known from more than 100,000 bird of paradise skins exported from New Guinea. This low incidence of hybrids is significant in view of the fact that social patterns are more favorable to hybridization in this group than in most birds, for there is no pair formation and care of young by both parents in most of the species. Even in the fishes, from which species hybrids have been reported more frequently than from other groups, Hubbs, Hubbs and Johnson (1943) in analyzing natural hybridization in suckers (Catostomidae) estimated that in those parts of the stream systems where both parental species occur, "perhaps one sucker in 100 is an interspecific hybrid." Under conditions highly favorable to failure of isolating mechanisms, Bailey and Lagler (1938) found recognizable hybrids comprising as high as thirteen per cent. of centrarchid populations. The most striking evidence that natural species hybrids are rare in animals comes from the work with Drosophila. In this group, in which natural populations have been sampled more extensively than in probably any other group of animals and in which many species have been crossed in the laboratory, Patterson (1947) reported that hybrids between only one species pair had been found in nature.

The scarcity of recognized natural hybrids between vertebrate species might be explained in several ways: (1) once isolating mechanisms have developed, they are so effective that the species do not hybridize in nature, or they hybridize so infrequently that hybrids are exceptionally rare, (2) too little work has been done to reveal the extent of natural hybridization, (3) hybrids are produced but are generally unrecognizable as such.

Anderson (1948) favoring the third explanation above holds that, "when hybrids do occur they usually perpetuate themselves, if at all, in backcrosses to one or the other parental species, and the mongrel nature of their descendants is not apparent to the ordinary biologist." He also emphasizes the fact, apparent also from the study of vertebrate hybrids, that natural hybrids are usually found in association with man-made disturbances of the environment. Anderson's explanation is that hybrids are generally able to survive only where the habitat has been "hybridized" due to human disturbance. This theory cannot be proved or disproved on what we now know about the survival value of different genetic combinations in nature in the vertebrates. An equally tenable hypothesis would be that hybrids are found in disturbed environments because the disturbance has lowered ecological barriers to interbreeding.

What is the Effect of Sympatric Hybridization?

The amount of gene transfer between sympatric, vertebrate species is difficult to evaluate. It is my opinion that the rare hybridization of sympatric species in nature accounts for very little transfer of genes from one species to another. The strongest argument comes from the fact that the morphological differences between most sympatric species are clear-cut ones. In the cases where hybrids have been reported, the parental species maintain their identity even though local, hybrid swarms may be formed. We recognize, of course, that the possibility of gene transfer, or introgression, exists in any case in which interfertile species hybridize. We know virtually nothing about the social relationships of natural hybrids to the parental populations, or about the survival value of hybrid genotypes in nature. The possible behavior of hybrids toward the parental species has been tested in one case in Peromyscus (Blair and Howard, 1944). The species maniculatus and polionotus, which are geographically isolated and which show strong mating preference in the laboratory, exhibit marked heterosis in the F_1. The hybrids pair indiscriminately with the parent maniculatus but show sexual isolation from the polionotus. In the hypothetical event of future overlap of range and of the breakdown of the mating preference bar to interbreeding, there could presumably be a transfer of polionotus genes into maniculatus, with little or no counter flow of maniculatus genes into polionotus. This assumes, of course, that the hybrids would survive in nature to sexual maturity.

If interspecific hybridization is as common in animals as Anderson (1949) believes it to be in plants, the mechanisms of reproductive isolation between species should tend to disappear. Most biologists agree that these mechanisms are genetic in origin. Interbreeding would tend to dissipate such isolating mechanisms between the parent species as mating preference and courtship pattern even if the original hybrids tended to perpetuate themselves principally in backcrosses to the parent species, as suggested by Anderson.

INTERBREEDING OF INTRASPECIFIC POPULATIONS

Morphologically differentiated populations exist within the limits of a single species in all vertebrate groups. In fact, the polytypic species has been recognized as such for many years by the students of some vertebrate groups. Recognition of the fact that the interbreeding (or hybridization if you prefer), as we see it in nature, of different infraspecific populations may come about in different ways has been a rather recent development. Mayr (1942, p. 99) distinguishes between what he calls primary intergradation and secondary intergradation in polytypic species populations. In the case of primary intergradation, contiguous, continuously distributed populations have differentiated morphologically into geographical races, which are connected by so-called zones of intergradation in which there is a steepening of character gradients. In the case of secondary intergradation, populations that have been separated geographically in the past without developing effective reproductive isolation have reestablished contact and now interbreed in the zone of overlap. There is a steepening of the character gradient in this case, also, in the zone of hybridization. The interbreeding of morphologically unlike populations and consequent gene exchange in both of these cases is called introgressive hybridization by Anderson (1949). I believe the general tendency in the literature is to call the first case introgressive hybridization of subspecies. The second case is frequently called introgressive hybridization of species because of the conventional taxonomic practice of treating differentiated, allopatric populations as full species. This is an artificial distinction if we accept the modern, biological definition of the species, because the populations showing secondary intergradation are demonstrating that they have not developed effective isolating mechanisms. A great majority of the cases of so-called introgressive hybridization in both plants and animals appear to be cases of either primary or secondary intergradation. For this reason, it is useful to review the dynamics of natural populations in respect to the origin of both of these types of interbreeding of populations.

Primary Intergradation

The differentiation into geographic races within a continuously distributed population apparently results from the combined actions of the forces of: (1) mutation, (2) selection, and (3) retarded gene flow due to the existence of ecological barriers (see Wright, 1931, ff.). The evidence from Peromyscus has been summarized by Dice (1940a) and by W. F. Blair (1950). Selection seems to be the most important single factor affecting the dispersal of mutant genes in the species population, and consequently affecting the gene complexes of the infraspecific populations, although population structure and pattern of distribution also have important effects. It is not too much of a generalization to say that the local gene complexes within a species population are a reflection of the environmental complexes within the range of the species. Where the environment changes gradually, there is gradual change in the morphological characters of the species to produce what the taxonomist would call a wide zone of intergradation. If the change in environment is gradual from one end of the species range to the other, morphologically quite different populations at the extremes of the species range may be connected by gradual clines in morphological characters, without any abrupt break in the character gradient. Where the environment changes abruptly, there may be an abrupt change in characters of the species. In this case, two well differentiated populations may exist contiguously and be connected by morphologically intermediate populations in the zone of abrupt environmental change.

Zones of primary intergradation have been studied more intensively in Peromyscus than in probably any other group of vertebrates. A broad zone of intergradation between *Peromyscus maniculatus bairdii* and *Peromyscus m. osgoodi* across North Dakota was found by Dice (1940b) to correspond to a gradual increase in elevation, decrease in amount of precipitation, increase in the arid character of the vegetation and increase in paleness of the soil. A narrow zone of intergradation between the well-marked subspecies *Peromyscus polionotus polionotus* and *Peromyscus p. albifrons* in western Florida has been intensively studied (Sumner, 1929a, 1929b, 1932). The albifrons occurs on pale-colored sands of coastal beaches and is replaced by polionotus on the dark-colored soils of the interior. The two forms intergrade in a zone only a few miles wide, inland from the coast. Much of the color difference between the two populations is due to the action of a single pair of alleles affecting coat pattern (W. F. Blair, 1944b). The dominant gene, acting to restrict distribution of pigment, and affected by a complex of minor modifying genes, predominates in the albifrons population. The polionotus population is almost or completely homozygous for the recessive allele that produces "normal" pigmentation. Selection in favor of adaptive color types on the very differently colored beach and inland soils can account for the origin and continued existence of these color populations. Haldane (1948) using our data, and making certain necessary assumptions, some of which unfortunately are not justified, calculated that the intensity of selection in the *Peromyscus polionotus* populations would have to be only 0.1 per cent. to account for the situation discussed above. It seems likely that the intensity of selection on the two soil types is considerably higher than this, and Haldane (*op. cit.*) believes it is probable that, "the light varieties were formed under the action of much more intense selection on the beaches." In this case, and in others studied in Peromyscus, environmental selection appears to be the most important factor maintaining the geographic races. A change in the environment in any part of the species range might favor extension of the range of one adaptive gene complex at the expense of another. Addition of organic material to darken the beach soils would favor extension of the polionotus type onto the beaches. On the other hand, an environmental change might change the conditions of selection so much that the characters of the local race would be changed simply due to the differential survival of mutants and to the shifting of existing, local gene frequencies. This change could come without any acceleration of gene flow from contiguous populations. Additional darkening of the inland soils would shift selection there to favor survival of the darkest-colored polionotus, but it would maintain or intensify selection against the albifrons type.

The physiological races of *Rana pipiens* reported by Moore (1949) also show adaptive differentiation of sub-populations within the species population. Northern populations differ from southern ones in such adaptive characters as embryonic temperature tolerance, rate of development, effect of temperature change on rate of development, egg size, and form of egg mass. These are adaptations to particular environments just as much as pelage colors and tail length are adaptations to particular environments in Peromyscus. The adaptations in Rana are principally adaptations to the physical environment, while those in Peromyscus are adaptations to the sum of the physical and biological environment. Another case has been analyzed by Heuts (1947) in the European stickleback (*Gasterosteus aculeatus*). Two sharply defined, physiologically adapted populations occur in Belgium, one in fresh water and the other in salt. The two populations differ in such morphological characters as plate number and body size and in physiological traits, the most important of which concerns chlorine elimination from the blood. The change from one type to the other is rapid, where the water changes rapidly from fresh to salty and brackish. Gene flow between the two populations is limited by selection against the adults and even more strongly against the eggs.

That the genotypes of infraspecific populations are principally the result of environmental pressures is probably more clearly recognized by botanists than by zoologists. The work of Turesson (1922), Clausen, Keck and Hiesey (1941, ff.), and Gregor (1939, ff.) goes beyond anything yet accomplished by the zoologists in demonstrating the adaptive character of geographic subpopulations of species.

If, as we believe, geographic races are molded by their environments—ecological trends of Dice (1940a)—the gene complexes of these races must shift continuously in response to local shifts in environmental conditions. Gershenson (1945) found cyclic, seasonal shifts in the frequency of black and agouti color types in the hamster (*Cricetus cricetus*). Dobzhansky (1943) has demonstrated such cyclic shifts in gene arrangements in natural populations of *Drosophila pseudoobscura*, and Dobzhansky (1947) and Dobzhansky and Levene (1948) have shown these shifts to be caused by natural selection. These findings all bear out the more indirect evidence from Peromyscus that the gene complexes of natural populations are molded by the environment, and they suggest a sensitive relationship between gene frequency and environment. Wright (1949) points out that, "a continual, kaleidoscopic shifting of the statistical characters is to be expected within any species that occupies, not too densely, a reasonably large range."

There is some objection to the use of the specialized term introgressive hybridization in reference to the long-recognized phenomenon of gene flow between infraspecific populations. The idea of the progression of one form into the other is implicit in the term introgressive hybridization if we interpret correctly the usage and implications of the term by Anderson (1949), Heiser (1949), Woodson (1947) and others. The idea of the movement of one form into another is not consistent with the concept that geographic races exist as environmentally controlled gene complexes. Just because two morphologically dissimilar forms interbreed freely in a zone of contact does not mean that the genes of either population will be distributed freely throughout the range of the other. The rate of gene flow between sub-populations of a species is controlled by many factors, including principally: (1) population density and structure, (2) pattern of distribution, and (3) rate of selection. Gene dispersal from one geographic race into another will be controlled largely by environmental selection. In other words, an equilibrium must exist between gene flow and selective elimination of immigrants to permit the origin and perpetuation of geographic races within a species population. A polytypic species nevertheless evolves as a single system, as Dobzhansky (1949a) points out, although its geographic races are adapted to the environments of their respective territories. Any gene or gene arrangement possessed by the species theoretically may be dispersed to any part of the species range. Whether it is so dispersed or not depends on ecological factors.

The rate of differentiation of adjacent sub-populations will depend, of course, on the level at which equilibrium between immigration and selection is reached. A case in point is that of the black-colored races of rodents on the Tularosa Malpais and other lava flows in the Southwest (see Benson, 1933; W. F. Blair, 1943b, 1947; Dice, 1939; Dice and Blossom, 1937). One species, *Peromyscus eremicus*, is represented on the Malpais by a highly variable population in which some individuals are highly melanistic and others are nearly or quite as pale as the populations occupying the limestone rocks of the nearby hills. A reasonable, although hypothetical, history of the evolution of this color race would be as follows. The lava flowed down the Tularosa Basin, possibly as recently as 10,000 years ago to cover an area about forty miles long and from one to six miles wide. As vegetation invaded the lava bed there was also an invasion of pale-colored eremicus from the limestone hills. Existence was probably precarious for the pale-colored mice on the dark lava rock, and high immigration pressure from the surrounding populations may have been necessary to maintain even a sparse population on the lava. Mutations toward a melanistic coat color presumably occurred in the lava-bed populations at the same rate as they had occurred in the parent population. Such mutations were now of distinct advantage to their possessors. The melanistic mutants showed a higher survival rate and consequently a higher reproductive rate than the non-mutants, whereas they had been selected against in the parent populations. A continued high rate of immigration has prevented the development of a uniformly dark-colored population on the lava bed. If this has been the history of the evolution of the dark-colored population, then differentiation has taken place against the pressure of continued immigration. The present frequencies of the color genes in the lava-bed population are apparently a reflection of the equilibrium between selection pressure and immigration pressure. We cannot discount the possibility that the genes for paleness of pelage color have existed in the lava-bed populations since the lava bed was colonized. Immigration pressure is indicated, however, as a contributing cause of the variability by the fact that the lava-bed population of eremicus is less isolated ecologically than are other rodent populations that show less variability on the lava bed. We cannot overlook the possibility that selection on the lava bed favors heterozygotes in a manner similar to that described by Dobzhansky (1948) from Drosophila. The differences between the dark and pale mice are apparently due to multiple alleles. Selection in favor of heterozygotes would not result in the elimination of either extreme from the population.

The effects of potential gene flow and of selection on adaptive differentiation of infraspecific populations has been studied in *Peromyscus maniculatus blandus* in southern New Mexico (W. F. Blair, 1947b). Allelic genes with major effects on pelage color showed adaptive differentiation in frequency (75 per cent. recessive gray on gray soil and 43 per cent. gray on red soil) at stations eighteen miles apart in a continuously distributed population. There was no differentiation in the frequency of these color genes on differently colored soils at stations four miles apart in a continuously distributed population. Populations twenty miles apart, on similarly colored soils and separated by a strong ecological barrier, showed no differentiation in the frequency of these color genes. Both ecological isolation and environmental selection affect the differentiation of these local populations. Isolation by distance, as little as eighteen miles in this case, coupled with selection, permits adaptive differentiation. Parallel selection in similar environments inhibits differentiation even when there is reduced gene flow between the populations.

Our second objection to the use of the term introgressive hybridization in reference to gene flow between infraspecific populations is that previous geographic discontinuity of the populations is implied, or even postulated. By emphasizing the presumed merging of what are assumed to be previously discrete populations, the proponents of introgressive hybridization tend to overlook the fact that zones of intergradation between subspecies are maintained by the balance between gene flow and selective elimination of immigrants into the respective populations. A case in point is Woodson's (1947) study of *Asclepias tuberosa*. His distribution map for the species shows an excellent example of what I would call primary intergradation of three geographic races. I doubt that the origin of these geographic races has been very different from that discussed above for geographic races of mammals connected by zones of primary intergradation. Woodson, on the other hand, postulates, "origin in isolation on the Paleozoic and early Mesozoic land masses Ozarkia, Appalachia, and Orange Island." There is no proof in his data for the conclusion that a certain leaf modification, "because of certain associated selective advantages, appears to be supplanting the ancestral race." We might just as well say that *Peromyscus maniculatus osgoodi* is swamping *Peromyscus m. bairdii* because the change from one to the other takes place over a wide area and because osgoodi is a larger and therefore presumably a more vigorous mouse.

Secondary Intergradation

Secondary intergradation, as stated earlier, has a history entirely different from primary intergradation, although it is not always possible to determine which phenomenon is involved when examining specific cases. As stated earlier, speciation usually seems to be initiated by the spatial isolation of a part of a previously interbreeding population. The isolated populations gradually come to differ in morphological and in other characters for reasons that need no review here. These populations will tend to acquire differences that will prevent their interbreeding if the separate populations should ever come to occupy the same region. That isolating mechanisms develop gradually is indicated by the fact that several different mechanisms usually act to prevent interbreeding of sympatric species (table 1). Spatial isolation is, of course, an isolating mechanism of great importance in itself. Its weakness lies in the fact that its effects can be cancelled by any shift in range that would bring the previously isolated populations into juxtaposition. If such a shift in range comes before the populations have acquired intrinsic isolating mechanisms, the two populations will interbreed in a zone of secondary intergradation, or one population might theoretically swamp the other.

The shifting of populations, both the spatial isolation of parts of previously interbreeding populations and the reestablishment of contact by previously isolated populations, apparently results principally from major, regional changes in environment. Present day distribution of species, of speciating allopatric populations, and of zones of secondary intergradation between previously separate populations must be interpreted in relation to Pleistocene climatic shifts. An excellent discussion of the relationship between present distribution of both plants and animals and Pleistocene climates is given by Deevey (1949), who takes the rather extreme position that, "nearly all well-studied cases of subspeciation and speciation point to the Pleistocene as the time of . . . previous isolation, and the occurrence of closely related forms in the same area is therefore attributed to post-Pleistocene alterations in geography and biogeography."

Isolation of fragments of warm-climate populations in Florida and Mexico was one of the major biogeographic results on this continent of the southward shift of cold during the Pleistocene glacial stages. The evidence from both plants and animals is well summarized by Deevey (*op. cit.*). The fragmentation of bird populations in other glacial-stage refuges on this continent has been discussed by Rand (1948).

If the rates of evolution and of spread are different in different species, the population pairs formed by Pleistocene isolation in Florida and Mexico should vary greatly in their stage of speciation. We should expect to find: (1) isolated allopatric populations that have shown little morphological differentiation, (2) isolated, allopatric populations that have differentiated morphologically but have not developed intrinsic isolating mechanisms, (3) allopatric populations that have differentiated morphologically and have developed isolating mechanisms, (4) populations that are now sympatric due to post-glacial spread after effective isolating mechanisms were developed, and (5) populations that underwent a post-glacial spread and established contact before they had developed effective isolating mechanisms. In this last situation we would expect to find secondary intergradation between the previously isolated populations.

East-west populations attributable to Pleistocene isolation in Florida and Mexico are common among the vertebrates (table 2), but in most cases our information about the biological differentiation as opposed to strictly morphological differentiation is sadly lacking. A representative case in which there seems to have been little morphological differentiation of the still-isolated populations is that of the gopher frogs, *Rana capito* of Florida and the Atlantic coastal plain and *Rana areolata* of the Mississippi Valley west into Kansas, Oklahoma, and Texas. An apparently disjunct population on the coast of Mississippi and Alabama known as *Rana sevosa* will possibly be found to be continuous with the Florida capito when western Florida has been adequately explored. A remarkably similar example is found in the atrox group of rattlesnakes (see Gloyd, 1940). The population known taxonomically as the species adamanteus has spread from its presumed Pleistocene refuge in Florida northward along the Atlantic coast and westward along the Gulf coast to the Mississippi Delta. The western population, treated taxonomically as the species atrox, extends westward from the border of the pine-oak forest in Texas to California. A third population, the species ruber, presumably originated under Pleistocene isolation in Baja California, overlaps the range of atrox in southern California without any apparent interbreeding. Numerous other examples could be cited to show that population fragments isolated in Florida and the Southwest have not yet reestablished contact. An additional example in the tree frogs is that of *Pseudacris ornata* and *Pseudacris streckeri* with a distributional relationship very similar to that discussed above. It is unknown whether the species pairs mentioned above have developed effective isolating mechanisms that would prevent their interbreeding when and if they ever come to have overlapping ranges. In one case at least, that of *Peromyscus maniculatus* and *Peromyscus polionotus*, the allopatric populations have acquired mating preferences that should prevent interbreeding in case of overlap of range (Blair and Howard, 1944).

In a good many cases, the previously isolated populations have spread until there is overlap of range, with the two populations maintaining their

TABLE 2

PRESENT RELATIONSHIPS OF SOME NORTH AMERICAN AMPHIBIAN, REPTILE AND MAMMAL POPULATIONS THAT WERE PRESUMABLY FRAGMENTED DURING PLEISTOCENE GLACIAL STAGES

Populations		Present relationships
Eastern	Western	
Rana capito—sevosa	*Rana areolata*	Spatially isolated
Pseudacris ornata	*Pseudacris streckeri*	Spatially isolated
Microhyla carolinensis	*Microhyla olivacea*	Overlap; reproductive isolation
Pseudacris nigrita	*Pseudacris clarkii*	Overlap; reproductive isolation
Bufo w. fowleri	*Bufo w. woodhousii*	Overlap; secondary intergradation
Crotalus adamanteus	*Crotalus atrox*	Spatially isolated
Pituophis c. sayi	*Pituophis c. catenifer*	Overlap; secondary intergradation
Peromyscus polionotus	*Peromyscus maniculatus*	Spatially isolated; reproductive isolation
Peromyscus gossypinus	*Peromyscus leucopus*	Overlap; reproductive isolation
Neotoma floridana	*Neotoma micropus*	Meet, do not interbreed

distinctness in the zone of overlap. The *Peromyscus leucopus—P. gossypinus* pair is a good example in mammals. The ranges of the eastern *Neotoma floridana* and the western *Neotoma micropus* meet in central Texas. The ranges of these packrats apparently do not overlap, nor do the two populations appear to interbreed. Among the amphibians, the eastern *Microhyla carolinensis* and the western *Microhyla olivacea* overlap ranges in eastern Texas and Oklahoma. In eastern Texas, the two populations have different calls and are somewhat separated by different ecological preferences, although both have been found breeding in the same pond. A. P. Blair (1950) has reported similar behavior of the two populations in eastern Oklahoma. Hecht and Matalas (1946) claim to have found intergrades between the two populations at one locality in southeastern Oklahoma and at one locality in southern Texas. If true, this would indicate that the isolating mechanisms between the two populations are still weak. The eastern population of the widely distributed *Pseudacris nigrita* meets the range of *Pseudacris clarkii* in eastern Texas, Oklahoma, and Kansas. In Texas, at least, these behave as good species, having different calls and showing no evidence of interbreeding in their zone of overlap. In the case of all of these population pairs, the circumstantial evidence indicates that an effective complex of isolating mechanisms was developed either during the period of isolation or possibly, as Dobzhansky (1949b) suggests, through selection when the two populations reestablished contact.

Interbreeding of the previously separate populations is to be expected in the case of the populations that reestablished geographical contact before developing effective isolating mechanisms. Well-authenticated cases of secondary intergradation are comparatively rare in North American vertebrates. There are probably two major reasons for this scarcity: (1) the interval between glacial-stage splitting of populations and subsequent spread was sufficiently long to allow specific differentiation (that is, acquisition of isolating mechanisms) as evidenced by the cases mentioned earlier above, (2) cases of secondary intergradation often cannot be distinguished from primary intergradation.

Cases of presumed secondary intergradation in several groups of European forms, following post-glacial spread, have been summarized by Mayr (1942). A few cases have been reported from North America, but some of these may be cases of primary intergradation. The frequently cited case of the flickers, the eastern *Colaptes auratus* and the western *Colaptes cafer*, which interbreed in a wide zone from Texas to British Columbia might be interpreted as either primary or secondary intergradation. Another frequently cited case, that of the bullsnakes (*Pituophis catenifer* and *Pituophis sayi*) now regarded as a single polytypic species could be a case of either primary or secondary intergradation.

At least one of the cases called species hybridization of toads by A. P. Blair (1941a) appears to represent a secondary contact and partial fusion of previously isolated populations. The eastern *Bufo fowleri* and the western *Bufo woodhousii* meet in eastern Oklahoma and Texas. In Oklahoma, "where the east-west topographic change is abrupt, as the transition from Ozark Hills to Osage Plain, the ranges of the two species meet, but few or no intermediates are found. Where change is more gradual (LaMine and Yuba) intermediate populations are found" (*idem*). The grassland-inhabiting woodhousii probably spread northward and eastward from a Pleistocene refuge in Mexico, following the retreat of the Wisconsin glaciers. The present contact permits interchange of genes along the line of secondary intergradation. There seems no reason to believe that the penetration of the genes of one population into the other will be any greater than that between two forms that show primary intergradation. Each form is adapted to a particular environment, woodhousii to grassland and fowleri to the deciduous forest, and selective elimination of non-adapted genotypes should act to maintain the adaptive characters of the two populations and to limit intermediates to the zone of transition from grassland to deciduous forest.

In *Peromyscus maniculatus*, the intergradation between grassland and forest forms in western North America is probably of the secondary type. This is an old species, as evidenced by its wide distribution, by its large number of continental and insular subspecies, and by the fact that it has given rise to other species around the periphery of its range (see Osgood, 1909; W. F. Blair, 1950). Two major ecological types exist within the species, and each type includes numerous subspecies. One is a short-tailed, short-eared, small-footed grassland form, while the other is a long-tailed, large-eared, large-footed inhabitant of forests. The forest form has a roughly U-shaped distribution, extending northward through the Appalachians, westward across the Canadian forests and southward in the mountains of western North America. The grassland form ranges northward from Mexico and occupies the central grasslands inside the U-shaped range of the forest populations. The two forms meet without interbreeding in Glacier Park, Montana (see Murie, 1933; Mayr, 1942). Clearing of the forest has permitted the grassland form to reach the range of the forest form in Michigan and New York (see Dice, 1931; Hooper, 1942; Hamilton, 1950). The two forms interbreed in some places along the eastern front of the Rocky Mountains and in other parts of western North America. Post-Pleistocene changes in the climate of central North America presumably forced the withdrawal of the forest from this area and permitted the northward spread of the grassland form from a refuge in Mexico.

Numerous cases of both primary and secondary intergradation are cited by A. H. Miller (1941) in the avian genus Junco. The populations of this genus are, in Miller's words, "almost without exception geographically complementary." In addition to well-marked forms showing primary intergradation, there seems to be a considerable number of previously isolated populations that have rejoined ranges before developing effective isolating mechanisms. Miller relies considerably on morphological differentiation in recognizing ten species among these geographically complementary populations. In spite of this taxonomic expedient, I would regard the interbreeding of many of these named populations as secondary intergradation of populations that were in incipient stages of speciation before reestablishing contact in their ranges.

Reestablishment of contact and subsequent secondary intergradation by previously isolated populations should reestablish a relationship similar to that existing between infraspecific populations showing primary intergradation. Each secondarily intergrading population will be adapted to its own environment, and the interchange of genes between such populations will be conditioned by the same factors of selection and of population structure that affect the interchange of genes between populations showing primary intergradation. The reunited populations will now evolve as a single system.

SUMMARY

Sympatric species are prevented from interbreeding by complexes of isolating mechanisms, and hybridization involves the breakdown of some of these mechanisms. Closely related species are frequently interfertile in the vertebrates, and continued segregation of related sympatric species is dependent on the effectiveness of the isolating mechanism complex. Hybrids between sympatric species of vertebrates are rare, and they usually occur where there has been a breakdown of ecological isolation. The amount of gene transfer between sympatric species of vertebrates is unknown, but it seems probable that it is negligible.

The origin and perpetuation of morphologically differentiated subpopulations—geographic races—are controlled by mutation, selection, and amount of gene flow. Local gene complexes are, in general, reflections of the local environmental complexes. An equilibrium between gene flow and selective elimination of immigrants must exist to permit the origin and continued existence of geographic races. The polytypic species evolves as a single system because of gene exchange, but each race is adapted to its own environment.

Fragments of species populations that have been spatially isolated, as by Pleistocene climatic shifts, may rejoin ranges before an effective complex of isolating mechanisms has been developed. Under such circumstances, the previously isolated populations will show secondary intergradation along the line of contact. The interchange of genes between secondarily interbreeding populations will be conditioned by the same factors of selection and population structure that affect the exchange of genes between geographic races that have not been spatially isolated. The reunited populations will now evolve as a single system. Populations of vertebrates showing all stages of incipient speciation, following Pleistocene fragmentation of ancestral populations, occur in North America.

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THE TAXONOMIC UNITS IN RELATION TO CYTOGENETICS AND GENE ECOLOGY[1]

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The history of the species question is one of the longest and most complicated in biology. I shall not attempt to recount it here, but I wish to deal mainly with the subject in its modern aspects, from a biological point of view. Having made extensive genetico-taxonomic investigations of the genus Oenothera over a period of many years (Gates, 1936) as well as briefer studies of many other plant genera, I should not be entirely unfitted for this task. There have been innumerable attempts to define species, but none of them have proved satisfactory or of universal application. Let me emphasize first that an inclusive definition of species which would apply to all groups of organisms is a will-o'-the wisp, which would be of no value even if attainable. How can one expect to apply the same concept of species to, for example, bacteria and mammals or, let us say, to Cyanophyceae and flowering plants? To do so, considering only the vast range of differences in complexity of these organisms, implies an acrobatic effort in semantics, without finding any tangible common denominator except that in every group of organisms there are many different kinds. Moreover, the generic and specific differences appear to be no less marked in a group such as the Cyanophyceae, without sexual reproduction, than in the Rhodophyceae where sexual reproduction rules.

When we consider the multitude of ways in which new inheritable forms can arise, as disclosed by the modern work in cytogenetics and in experimental ecology, the futility of trying to comprise them all under one formula begins to appear. There is nevertheless one school of geneticists who have attempted to erect reproductive isolation as *the* criterion of species. This attitude, and the chaos to which it leads in animal species, has been criticized elsewhere (Gates, 19~~4~~38). In short, it induces Dobzhansky, a leading exponent of this view, to be an ultra-splitter in one genus and an ultra-lumper in another. Dobzhansky (1946) describes a "new species" of Drosophila, *D. equinoxialis,* which will not cross with *D. willistoni* although there is a "virtual lack of morphological differences." There is no difference in the male external genitalia, the chromosomes are alike in both forms, but there is a mean difference in body size although the curves of size variation broadly overlap. Average individuals cannot be sorted into one "species" or the other; but because the reproductive isolation is virtually complete it is affirmed that there is "no reasonable doubt that these forms are full-fledged species rather than races of the same species." That morphologically identical forms are different species because they will not intercross, involves a contradiction in terms. The science of taxonomy is founded on the existence of visible differences by which forms can be classified. These Drosophilas are not full-fledged species, but if they continue in physiological isolation they may become so in ten thousand or fifty thousand years. But that is no justification for calling them species now. Microspecies would be a more appropriate name for them.

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[1] Invitation paper presented at a joint meeting of the four Sections, Genetics, Cytology, Experimental Taxonomy and Taxonomy of Phanerogams, at the VIIth International Botanical Congress, Stockholm, July, 1950.

Drosophila persimilis and *D. pseudoobscura* are a similar pair of forms. Although partly sympatric and morphologically identical, reproductive isolation "appears to be complete in nature" through the cooperation of several isolating mechanisms. On this ground alone they are affirmed to be "unquestionably species." In opposing this view, Huxley (1940, p. 16) says, "When (Dobzhansky) tries to define the species-level as that stage in taxonomic differentiation after which fertile interbreeding is impossible, he goes far beyond the facts... it is impossible to insist on infertility as the sole criterion of species." One of the greatest defects of this view is that it ignores the aims and methods of taxonomy and seeks to make taxonomy subservient to a condition—sterility—in genetics, by raising it to the importance of a universal principle.

Muller (1942) is equally emphatic in stating (p. 96) that "in this genus (Drosophila) at least no sharp line can be drawn between sub-divisions of one rank, such as races or sub-species, and of another rank, such as species."

In a subsequent paper (Burla *et al*, 1949), Dobzhansky and his colleagues find that the four forms, *D. willistoni*, *D. paulistorum*, *D. equinoxialis* and *D. tropicalis*, are all sympatric in parts of Brazil. Although individuals of these microspecies could be distinguished with difficulty only in fresh material, even by those trained in Drosophila work, yet they are said to show varying degrees of sexual isolation and to be recognizable by the disc patterns in the chromosomes of the larval salivary glands. Only excessive zeal in applying the single criterion of intersterility would lead anyone to call them species rather than microspecies.

"Intersterility may in fact arise at any stage in the process of speciation and is frequently the first stage rather than the last in the production of a new species" (Gates, 1938). Several cases of single factors or complementary genes producing intersterility are now known and may be briefly cited here. Hollinshead (1930) described the first case, in Crepis. Some plants of *C. tectorum* were found to be heterozygous and some homozygous for a single recessive gene which, in crosses with *C. capillaris*, produces seedlings which die at the cotyledon stage. The same lethal effect was produced in crosses of *C. tectorum* with *C. bursifolia* and *C. leontodontoides*. But this factor had no effect in crosses with *C. setosa* and *C. vesicaria* *taraxacifolia.* This gene for intersterility was evidently widespread in parts of the distribution of *C. tectorum*, and there are indications of a similar interspecific lethal in certain other species of Crepis. Hutchinson (1932) found "crumpled," an abnormal sterile or semi-sterile type arising in *Gossypium Nanking* var. *soudanensis* × *G. arboreum* var. *sanguinea.* This was due to complementary factors, *A* present in two strains of *G. Nanking* from the Sudan, and *B* in seventeen varieties of *G. arboreum, G. Nanking, G. herbaceum, G. obtusifolium* and a strain of *G. Stocksii* from Sind. In some crosses the presence of *A* and *B* produced shrivelled or aborted embryos, but modifying factors in Asiatic cottons affected the expression of the crumpled character and improved its viability. A more extreme lethal in *Hutchinsia alpina* produces empty seeds in crosses with *H. brevicaulis* (Melchers, 1939). The gene which prevents fertile crosses with *H. brevicaulis* is present in *H. alpina*, south of Innsbruck, but other races of *H. alpina* were fully fertile in crosses with *H. brevicaulis* from the Dolomites. A race homozygous for the intersterility gene must have arisen through inbreeding.

It is well known that species crosses in Oenothera frequently produce a non-viable type which dies in the seedling stage. Several such cases are cited by Gates and Catcheside (1932). Sears (1940) concludes that *Triticum monococcum* contains a dominant gene which is lethal in reciprocal crosses with *Aegilops umbellulata*, and Vavilov recognized such lethals in several crosses between European and Indian soft wheats. Dobzhansky (1941) refers to certain other cases. He regards these single genes as "merely extreme examples of the interdependence of genes composing a balanced genotype" (p. 279), but in any case the evidence shows that single genes may introduce intersterility among forms which are phenotypically indistinguishable. Patterson and Griffen (1944) described a sex-linked gene in *Drosophila texana* and *D. montana* which has no effect in its own species, but the montana egg fertilized by an X-bearing texana sperm produces an inviable female zygote. Here also complementary genes are involved, and a similar condition is present in the cross *D. mulleri* × *D. aldrichi* ♂. Thus single or complementary genes in nearly related forms can produce intersterility and prevent gene interchange.

When the matter of species is considered in its time aspect, from the point of view of palaeontology, it becomes clear that intersterility between contemporary evolving forms is incidental, depending on many chance vicissitudes but more especially on (a) chromosome translocations, deletions, reversals, and other changes, (b) amphiploidy, in which an interspecific hybrid doubles its chromosomes. The former conditions are found equally in plants and animals, whereas the latter occur mainly in plants. The evidence indicates that translocations, leading to linkage groups of genes and to intersterility, are universal in sexual organisms, although their frequency is higher in some plants than in others.

Thus contemporary species of a genus may or may not develop intersterility, or may develop it to very different degrees. On the other hand, when a species gives rise to one or more other species in geological time, it is obvious that there has been continuous fertility between the mother and daughter species throughout the transition period; except in the case of amphidiploids, where two parent species are involved in producing a stable new species within a short period after contact and crossing. When we contemplate the various other ways in which a condition of sterility can arise within a species, as by the formation of self-sterility genes or apomicty, it becomes clear that intersterility is an incidental phenomenon in evolution. It may arise early or late or not at all in the process of speciation.

There are whole genera or large subgenera of plants in which the species are nearly all interfertile. These probably include the raspberries, Rubus (Idaeo-batus), and Crataegus as well as Cypripedium[2] and many others. In Oenothera (Euoenothera) some 70 species appear to be all interfertile. Intercrossing seldom occurs between them in nature because most of the species of Oenothera are self-pollinating in the bud, before insects can reach the flower. Although these species are highly heterozygous, the catenation of the chromosomes, together with the presence of balanced lethals, prevents genetic segregation, so that successive generations breed true. This condition of chromosome catenation is no longer unique but is now known in many plant families, although its genetical effect is not necessarily the same in these genera. Not only in Gaura and Godetia and other genera of the Onagraceae but in *Hypericum punctatum* (Hypericaceae), Polemonium (Polemoniaceae), Aucuba (Cornaceae), Datura (Solanaceae), Paeonia (Ranunculaceae), Humulus (Cannabinaceae), *Campanula persicifolia* (Campanulaceae), Pisum (Leguminosae), Rosa (Rosaceae), Rumex (Polygonaceae), *Rhoeo discolor* (Commelinaceae) and Anthoxanthum, Avena, Briza and Zea (Gramineae) among flowering plants, and also in the Gymnosperms, Taxus and Cephalotaxus. The conditions which limit, without excluding, intercrossing range from self-pollinating mechanisms, such as that in Oenothera or wheat, and cleistogamy, to geographic isolation. They lead to every conceivable degree of partial isolation. It is clear that without isolation in some form speciation cannot take place (except again, in the case of amphidiploids and also in the geological sense where one species gives rise to another as descendant).

CHANGES UNDERLYING SPECIATION

From such facts as those already mentioned we may derive the view that in speciation there are two main streams of change: (a) gene mutations which alter the phenotype, and (b) chromosome changes such as translocations, which generally have no effect on the phenotype but which alter the gene linkages and also introduce intersterility. The existence of these two independent categories of variation results from the fundamental fact that every significant cell has a nucleus, the elements of which can undergo either structural or physiological mutations. Karyotype evolution can thus be largely independent of phenotype evolution, although both take place in the same organisms. It has been pointed out elsewhere (Gates, 1950) that although man in the past has selected only for the phenotype while nature selects for the whole organism at every level of structure, yet this phenotypic selection has resulted in changes of chromosome organization as well. There is no reason to suppose that any necessary relation exists between the frequency of gene mutations and the frequency of chromosome changes such as translocation. Indeed, the evidence indicates that while the former are most readily produced by X-rays and other forms of radiation, the latter often result in nature from excessive temperature effects or humidity in seeds, which cause the chromatin threads to adhere and break. In some plant genera with stable nuclei, such as Ribes, Cistus, Carica, Philadelphus and Streptocarpus, the evolution of many species has occurred without any change in number or marked visible alteration in the set of chromosomes. Reciprocal translocations and small deletions may nevertheless have taken place. In the genus Lilium, in which 45 species are known to have twelve pairs of chromosomes (with a few extra fragments in certain species) the only visible differences are in the number and position of the nucleolar loci (Stewart, 1948). In other genera, such as Crepis (Babcock, 1947), the nucleus has been in an unstable condition and many changes in the karyotype, such as alteration in the length of particular chromosome arms, diminution in chromosome number through a form of translocation, and decrease in size of all the chromosomes, have taken place.

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[2] In a letter of C. C. Hurst, he wrote in 1896 (R. Hurst, 1949), "with regard to the limits of species, I once fondly thought that fertility and non-fertility would be the true test of species...but my hopes have been sadly dispelled of late, since my experience with orchids." He crossed *Cypripedium barbatum* successfully with 24 distinct species out of some 30 cultivated in gardens. "All are distinct in every way and could not be termed varieties merely." There are two known cases in Orchids in which a hybrid has been built up from crosses made between four genera!

These two streams of change, nuclear and phenotypic, occurring in the same lines of descent in a species, will be equally subject to the forces of natural selection. There is evidence that in certain strains of Drosophila, individuals which are heterozygous for a certain translocation have a selective advantage over others in the population. This advantage may vary with temperature, thus leading to cyclic changes in the relative frequency of such individuals during different seasons of the year. Similarly it has been shown (Nabours and Stebbins, 1947) in the grouse locust, *Apotettix eurycephalus,* that a gene which is lethal in the homozygous condition produces viability superior to normal in individuals which are heterozygous for this gene. When present in double dose, the individual dies four days before hatching time, whereas a single dose produces individuals which are more vigorous, better feeders and better breeders than the normal. Gustafsson (1946) describes and cites numerous similar cases in both plants and animals, where an individual heterozygous for a lethal gene has superior viability.

Geneticists and taxonomists appear to be agreed that, although type specimens are of course necessary, a species is a dynamic system consisting of a population of living individuals. It is necessary to remember also that time enters as a fourth dimension into the concept of species.

We must then think of a succession of living populations as making up the species, until it finally in the course of time becomes another species or breaks up into two or more specific entities. The consideration of populations leads to other aspects of species on which there is less general agreement. In considering populations of Vaccinium, Camp (1942) says, "It is not within the province of the systematist to decide whether a population is worthy of being recognized as a species solely on the basis of its manner of origin; his primary problem is to decide whether it is an agrestial and self-sustaining population morphologically sufficiently distinct from others that it merits this rank." And he recognizes that in this decision some judgment must be used.

Camp also finds it necessary to distinguish between phyletic divergence (basic evolution) and "mere speciation," but such a distinction will probably be obscure in many cases. He believes, no doubt rightly, that many polyploids are outside the stream of basic evolution, but it can scarcely be held that all fundamental evolution is at the $2n$ level. For example, the whole group Pomoideae, successful fruit trees, have a derived basic chromosome number $n = 17$, different species being $2n$, $3n$ and $4n$. Similarly, the large and successful genus Acacia is based on a derived number, 13, having both diploid and tetraploid species. Acer also, a widespread and successful genus of temperate trees, has 13 as basic number, 18 species being diploid, three tetraploid, and in one (*A. rubrum*) individuals have been examined with $6n$ and $8n$ (104) chromosomes. On the other hand, *Houttuynia cordata* with *ca.* 96 chromosomes is probably at least octoploid and is clearly an isolated survivor, due for future extinction. Similarly Welwitschia, a relict Gymnosperm which is nearing extinction in the desert of southwest Africa, has chromosome counts of 42 and 84, the latter number being 12-ploid on the basis of 7, the basic number in the related genus Ephedra. The optimum degree of ploidy is evidently higher in some genera than in others, and many factors will determine this optimum.

In a further analysis of the nature of species, Camp and Gilly (1943), in an important contribution, recognize thirteen different kinds of populations or species, according to their manner of origin. "It is the genetic structure of a species which makes it a recognizable unit." They go on to say, to "make so broad an interpretation of the species as to include in it all forms which can be shown to be capable of interbreeding and producing fertile offspring, would lead to quite as great an absurdity" as to name as a species every form differing by a single character capable of vegetative or sexual reproduction. Among the kinds of species which they recognize are the homogeneon, a population in which all the members are interfertile and genetically and morphologically homogeneous—a relatively rare condition. Self-explanatory terms are euploidion, alloploidion, cleistogameon, heterogameon (heterozygous species, structural hybrids, breeding true, as in Oenothera), agameon. The micton consists of hybrids of two or more species, all interfertile. The rheogameon (syngameon or Rassenkreis) consists of interbreeding subspecies between which there is more or less "gene flow." Those who promulgated the International Rules of Botanical Nomenclature in 1935 still adhered to the ancient doctrine that hybrids between species, with but few exceptions, are sterile. It is to be hoped that we have now heard the last of this attempt to equate interfertility or intersterility with species. In general, Camp and Gilly recognize two kinds of species, those which contain accretional elements and those which are of concretional nature (containing subspecies).

SUBSPECIFIC CATEGORIES

This brings up in acute form the question of the treatment of subspecific categories. Without entering into detail on this subject one may point out that in the case of hybrid swarms any complete nomenclature would have to recognize almost every individual as different from every other. If the population is the unit, then the types within a mixed population should not receive Latin names, but there are exceptions to this rule. In entomology it is suggested (Hatch, 1946) that only populations merit a specific or intraspecific name, (the matter of naming being determined by usefulness and expediency), and that color varieties should receive common but not Latin names. The numerous color patterns in some species of insects renders this problem more acute for entomologists than for botanists, by whom such trivial names as *albida, inerma* or *nana,* are frequently used.

That the treatment of hybrid swarms is an important problem is shown, for instance, by the work of Allan (1940) in New Zealand. Cockayne and Allan recorded 491 hybrid groups, and Allan cites many cases of fertile species hybrids and hybrid swarms in the New Zealand flora. He separates them into pauciform and multiform. Later evidence will show that intercrossing, even over long periods, does not necessarily merge the species which intercross. As a widespread condition in nature, hybridization has played an important part in evolution, but the basic material for change has been supplied by gene—mutation and chromosome rearrangements. The frequently reticulate relationships between the characters of Angiosperm families and between smaller units probably involve in part former hybridization.

For units within the plant species the terms subspecies, ecotype, variety and forma have probably been in use most frequently. Camp and Gilly recognize that in practice many species are composed of subspecies. The term ecospecies can be of much value in the analysis of the nature of species, as Clausen, Keck and Hiesey (1939, 1940, 1948) have shown. Continuing and extending the methods of Turesson, by transplanting clones of the same ecotypes of many species to gardens with very different climatic conditions, they consider that ecotypes → ecospecies[3] → coenospecies → comparium, represent stages in an evolutionary sequence. They find no genetic barrier between ecotypes but they recognize (1939) that "There actually is a natural reason for the fact that although crossings constantly take place, the species have remained remarkably the same for centuries, as evidenced by herbarium specimens, and even for ages, as shown by geological records." They also agree that "it is impossible to produce definitions that will cover all differences between systematic units, because species are in all stages of evolution." Transitions occur between all the categories mentioned above; but barriers, internal (hereditary) or external (environmental), are the basis of classification. The genetic barriers between coenospecies are regarded as absolute, while a certain amount of gene flow takes place between ecospecies. Such an exchange may give an appearance of parallel variation in related ecospecies. In such genera as Salix or Viola there may be 20—30 ecospecies in one coenospecies. When experimental evidence is lacking, the terms subspecies, species and species-complex will take the place of the ecological terms. But ecospecies can only be determined with certainty by careful transplant experiments. The analysis of many species from this point of view must await larger facilities than at present exist.

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[3] Valentine (1949) proposes the following subdivisions: g-ecospecies having the same chromosome number; a-ecospecies with different chromosome numbers; caryotypes, a group forming polyploid components of an ecospecies.

This method is chiefly valuable in showing how precisely different ecotypes of many species, growing for instance at different altitudes, have become adapted to the climatic and edaphic conditions. California is a very favorable region for such experiments. A transect of 200 miles across Central California, from the seashore to the Coast Range, the San Joaquin Valley and the Sierra Nevadas above the tree line, gives an unsurpassed variety of climatic and altitudinal conditions. Clausen, Keck and Hiesey (1948) have applied this method to the genus Achillea. The Old World *A. millefolium* L. is hexaploid ($2n = 54$) and widely introduced in America. *A. borealis* Bong., also hexaploid, extends from the Aleutian Islands and Alaska down the coast to Southern California. Three subspecies are recognized, *arenicola* on the coast from Oregon to California, *californica* in the foothills of California, and *gigantea* in the San Joaquin Valley. *A. lanulosa* Nutt. is tetraploid ($2n = 36$) and native from New England to near the Pacific coast, where it meets *A. borealis.* Where these species meet they can only be distinguished by their chromosome number, but it is considered best to retain them as species because of their geographical distribution. The two native American forms are separated by trivial characters of leaf cut and vesture. The distribution was determined by 108 chromosome counts made by Lawrence (1947). A single location in California was octoploid.

The authors collected Achillea seeds from 80 localities, grew 60 seedlings from each, and selected 30 of these showing the entire range of variation, for clonal division. The clones were transplanted to gardens at Stanford near sea-level, Mather in the Sierras at 1400 m., and Timberline on the eastern Sierra slopes at 3000 m. In this way a surprising degree of genetic diversity was disclosed. "Almost every type of climate (in California) except the true desert has its race of Achillea," each being particularly well fitted to its native climate both in form and in developmental processes. Climatic, edaphic and biotic races are involved, the climate largely determining the other two. Each ecological race approaches an equilibrium with the environment, variations in the organism being balanced against natural selection acting through environmental factors. The variation in size alone is from lush valley forms to dwarf alpines, but neither will survive in the habitat of the other. The dynamic equilibrium of each local ecological race is nevertheless somewhat flexible, through the morphological and physiological diversity of its component individuals, scarcely two of which belong to the same biotype. There are at least eleven diverse climatic races along this 200 mile transect, each having a vast amount of genetic variability. In *Antirrhinum majus* Melchers (1939) has described a series of ecological mutants, heroina (tall), eramosa (unbranched) nana (dwarf), alpina (branched from the base), matura (with early flowers) and densiflora (with many grouped flowers). These, as well as Turesson's earlier work, show how ecotypes can have arisen through mutation.

In a similar study of the Californian *Potentilla glandulosa* Lindl., Clausen (1949) has used not only transplantation but crossing experiments. Many diverse ecotypes are found belonging to 11 or 12 distinct subspecies and about 8 climatic races in a transect of Central California. Although morphologically distinct, all are completely interfertile, having $n = 7$ chromosomes. In the Coast Range is subsp. typica; in the foothills of the Sierras *P. reflexa* Greene is reduced to a subspecies; *P. Hanseni* Greene occurs in meadows at higher altitudes; and *P. nevadensis* S. Wats. is another subspecies at alpine levels.

In transplants to the same three stations, typica and reflexa die at Timberline, while Hanseni survives at all three but develops best at Mather. Crosses between these three subspecies give an F_1 which is vigorous and fertile. In the F_2 for example, of typica × nevadensis, every recombination occurs. These were cloned and grown for 7 to 9 years at the three stations, and 20 F_3 progenies were produced. From a detailed analysis of the F_2, 60–100 genes were estimated to be involved. There were several genes (usually 2–4) for each character, and the physiological as well as the morphological characters were thus graded. The petal color ranges from white to yellow, and five pairs of genes for color were estimated to be present in crosses between the foothill and the alpine form. The F_1 had a wider tolerance than the parents, and could survive at all stations. All kinds of growth patterns were produced through gene exchange, including "super-alpines" which could thrive best in alpine conditions. The physiological and morphological characters were inherited independently so that some physiological alpines have the morphological traits of the foothill forms. Some recombinations were weak at all stations while some were successful in all, showing wider tolerance than any wild form. The ecotypes are thus as diverse as well recognized subspecies, but they are determined by many genes. Crosses between them, which are rare in nature, release new evolutionary possibilities.

I have referred to this work in detail because its carefully planned experiments, carried out on a large scale, throw much light on the nature of specific and subspecific variability. The three main forms, although morphologically distinct, are physiologically adapted to different habitats, and although fully interfertile rarely cross in nature. This is a typical borderline case where opinions will differ as to whether the three forms should be ranked as species or subspecies. The lumpers and splitters will differ on this point. Turrill (1936) cites a clear case where two species which readily intercross are prevented from merging by their different ecological adaptations. *Silene alpina* grows on screes in the French Alps, *S. Cucubalus* in meadows and woodland-edge. Seeds of *S. alpina* collected 50–100 m. above the meadows yielded 12 per cent. of hybrids, and in gardens they cross reciprocally. But they are kept separate by the physiographic, edaphic and biotic differences in their habitats.

The Case of Crepis

In his world monograph of the genus Crepis (already cited), based on thirty years cytogenetic, geographic and taxonomic work with the genus, Babcock (1947) disagrees with the point of view of Clausen, Keck and Hiesey that classification as species or subspecies should be based primarily on intersterility. He points out that the consensus of plant systematists is against it. He cites the case of *C. foetida, C. eritreënsis* and *C. Thomsonii*, which are highly interfertile but geographically isolated. He further finds it impossible to apply the concepts of ecotype, ecospecies, coenospecies and comparium in the genus Crepis. With a world background, the comparium (species which intercross to produce sterile hybrids) would contain species from many different sections of the genus.

In this extensive monograph the subgenera of Bentham and Hooker have been discarded as not conforming with the natural relationships of the species. Instead, the 196 species recognized (including 33 described as new) are divided into 27 sections. The genus is monophyletic except two sections which originated through hybridization with the ancestors of different genera. Evolution in the genus has been from species which are "robust, rhizomatous, mesophytic perennials with large simple fruits and short-lived seeds," to "small, delicate desert annuals, with their extreme precocity, ephemeral fibrous taproot, tiny fruits with a filamentous beak, and seeds of longer viability." In the genus, 143 species are perennial, 40 annual, the latter certainly derived, as is the taproot from the rhizome. Nodding versus erect heads, and the presence or absence of paleae on the receptacle, are each determined by a single gene.

The genus is probably derived from Dubyaea (with $n = 8$ chromosomes). In Crepis there has been a diminution of the chromosomes by steps, from $n = 6$ in primitive species to 5, 4 and finally 3 in the most advanced, one section alone being transitional, with $n = 7$. "Each step in decrease occurred independently of other steps and on several different occasions" (Babcock, 1947, p. 146), that is, these changes have been repeated and parallel. The reduction from 5 to 4 occurred at least four times, 58 species now having $n = 4$. This reduction in number has been accomplished by nonhomologous reciprocal translocations involving the loss of a kinomere and a chromosome fragment. Other translocations account for the increasing asymmetry in the length of chromosome arms. There is in addition a progressive decrease in size of all the chromosomes, which is correlated, at least in part, with diminution in size of the plant.

These lines of evolution have occurred in the Old World. The North American group (Section 15) have as basic number $n = 11$ chromosomes. They must have arisen as amphidiploids from Asiatic species having 7 and 4 or 6 and 5 chromosomes respectively. This group, confined mainly to Western North America, has also developed higher polyploidy (up to $2n = 88$) as well as apomixis, whereas very few of the Old World species are polyploid and none are known to be apomictic. Change in chromosome number in the diminishing series (e.g., $5 \rightarrow 4$) is not necessarily accompanied by much phenotypic change. However, it provides the basis for differentiation of species by gene mutations, which has been the chief agent of change in phenotype. The fact that 59 per cent. of the species of Crepis overlap with related species in their distribution shows how important nuclear changes have been in producing isolation. At the same time 133 of the 196 species are listed as endemic.

The net result of all the experimental work in cytogenetics and ecology is thus to show that while intersterility is an important criterion in determining taxonomic units, its application as a universal criterion would result in chaos, because intersterility is itself so sporadic in its development.

There is much diversity of opinion regarding the relative value of the concepts "subspecies" and "variety." Both terms, but especially the variety, have undergone changes of usage. R. T. Clausen (1941) strongly advocates following the custom of zoologists and using the subspecies simply as a trinomial attached to the specific name. This method appears to be not entirely in harmony with the needs of taxonomic botany. It would relegate the variety to its original more trivial use, as in horticulture and agriculture. There seems a place for both terms, *subspecies* being applied to forms which show more complex differences in correlation with the factors of geographical, ecological and physiological isolation, *variety* to differences of less significance.

SPECIES CRITERIA

To return to the question of the delimitation of species, most naturalists who have had wide experience in the taxonomy of any group of organisms appear to have reached finally the view that there is no criterion for a good taxonomist except that he have wide knowledge of his particular group, and good judgment. Thus Darwin wrote that "the opinion of naturalists having sound judgment and wide experience seems the only guide to follow," and Tate Regan (quoted in Gates, 1948, p. 378), an authority on fishes, came to a similar conclusion. Hitchcock (1925), a leader in the taxonomy of grasses, wrote, "The term (species) is difficult to define with precision because a species is not a definite entity but a taxonomic concept. Where a line shall be drawn is often a matter of convenience in classification." In cases where two populations are connected by scattered intermediate individuals, "Botanists will differ as to whether we have here two species with intermediate individuals, one species with two varieties, or subspecies, or one variable species....the actual delimitation of a species is a matter of judgment and experience." Babcock (1947, p. 34) writes in the same vein, "decisions concerning just how much difference between populations shall be sufficient for the recognition of a new species had best be left to the judgment of biosystematists who specialize in the various fields of organic life."

Harland (1936), after referring to the definitions of Ostenfeld, Vavilov, Hitchcock, Baker and Calman, concludes with the general consensus of opinion that it is not possible to define species in all cases, and that any attempt at definition should be in as general terms as possible. From his experience especially with the genus Gossypium he concludes that if two species or even genera can be crossed and produce fertile offspring there is always evidence that "either identical or at least homologous genes lie in homologous loci in the species involved." In six species of Gossypium he finds allelic relationships in all the genes examined and calls them "species alleles," but he regards the various modifiers as species differentials. His species alleles are thus not the same as the interspecific genes of Lamprecht (1948). The species differ not only in their main gene alleles but also in their complexes of modifiers, which are of primary importance from the taxonomic point of view. Gene substitution has then been the main mechanism of species differentiation in Gossypium through isolation.

There will doubtless always be lumpers and splitters according as the individual is more impressed by the resemblances or the differences between organisms involved. This is partly a matter of temperament, and the two activities involved are largely complementary. The splitter recognizes numerous smaller units, which the lumper combines so as to show their relationships. The activities of the splitter are necessary in connection with the genetic, cytological and ecological analysis of the subunits composing many species. The synthesis of the lumper is equally useful in reaching an understanding of the evolutionary history and relationships of any group of organisms.

ISOLATING MECHANISMS

All geneticists and most taxonomists will probably agree that the role of isolating mechanisms is fundamental in the differentiation of contemporary species. Stebbins (1942) has ably discussed this subject and expressed views which are, in the main, in agreement with those developed in the present paper. He recognizes that internal isolating mechanisms are genetically independent of morphological (phenotypic) changes, and that an understanding of them depends on knowledge of the many types of external and internal isolating mechanism which exist. He points out that geographic isolation does not necessarily lead to phenotypic divergence, and cites from the work of Fernald the striking cases of *Symphoricarpus foetidus*, *Cypripedium arietinum* and three species of Polygonum, which are identical in the eastern United States and in eastern Asia although they have been thus widely separated since the mid Tertiary. Equally striking cases are cited in Catalpa and Platanus. *P. orientalis* of Asia Minor crosses with *P. occidentalis* of the eastern United States to produce the London plane, frequently called *P. acerifolia*, which is fully fertile and exceptionally hardy, the chromosomes of the hybrid pairing normally in meiosis (Sax, 1933), its seedlings segregating in F_2. This genus goes back to the Cretaceous and was formerly distributed over nearly the whole northern hemisphere, many fossil species being recorded from Cretaceous and Tertiary deposits. These two surviving species must have been geographically separated for millions of years, yet the number of chromosomes ($n = 21$) in both species indicates either hexaploidy[4] or an origin through amphidiploidy, and the chromosomes appear to be of uniform size except for one possible smaller pair. The specific differences are imperfectly described but they pertain to every part of the tree. The number of gene pairs involved is roughly estimated as follows: habit of the tree (2), bark (1), hardiness (1), leaves (2), stipules (4), petioles (3), inflorescence (1), calyx (1), petals (1), making a total of at least 16 genic differences. It would seem that in this primitive Angiosperm genus at any rate a large amount of differentiation has taken place without any loss of interfertility. The florets are of extremely simple structure although their aggregation into a head is a specialization, but this has happened independently, e.g., in the genus Parkia of the Leguminosae as well as in the Compositae. Such cases lead one to doubt whether there is any limit to the number of gene pairs for which an organism may be heterozygous without impairing its fertility.

As regards Paeonia, which he has studied extensively, Stebbins (1942) believes the evidence "is definitely against the assumption that interspecific sterility results chiefly from gross structural changes" in the chromosomes. However, Paeonia, like Oenothera, has indulged in the form of chromosome interchange which leads to complete catenation and is not accompanied by intersterility. He thinks that intersterility in general has been built up by many small changes the nature of which is unknown. He finds that in various cases "The visible characteristics which differentiate the species and the sterility barriers which partly isolate them segregate quite independently of each other." In rice, on the contrary, he points out that some races which are very different morphologically are interfertile and some races which closely resemble each other are intersterile. This is exactly to be expected if, as I have suggested, chromosome changes leading to intersterility, and gene mutations in the phenotype, are independent in their origin and effect. Yet, as Stebbins states (p. 230) that "there is no direct correlation between the genetic isolating mechanisms which produce hybrid sterility and the genetic changes which make species visibly different," it appears that very little difference remains between his views and mine. Later, (1945), he appears in places to have succumbed to the view we are combatting, that intersterility or isolation factors can be fully equated with species. However, we assume that we would not agree with Dobzhansky in describing any forms which do not cross as new species, regardless of the absence of adequate phenotypic difference.

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[4] The three related families, Magnoliaceae, Trochodendraceae and Cercidiphyllaceae have $n = 19$, one species being tetraploid on this basis. This number 19 is probably amphidiploid in origin, indicating that, although the flower type is primitive among Angiosperms, the chromosome number is "advanced." This and other evidence increases the pressure to regard the Angiosperms as having a polyphyletic origin.

Further evidence that a great amount of differentiation between species can occur by gene mutations without loss in fertility is found in the work of Baur (1932) on Antirrhinum. He collected Antirrhinums all over Spain and the Western Mediterranean region. He found (1) a few local sharply isolated colonies representing our well-characterized species, (2) a series of nine large *Formenkreisen*, each composed of innumerable local races, generally of one type but connected by series of intermediates. The plants in some of these colonies were very heterozygous. *A. majus* grew wild in Southern and Middle Italy, Sicily, Algeria, Morocco, and the Iberian peninsula. Three genes of *A. hispanicum* were identical with those of *A. majus*. Baur concluded that over a hundred genes segregated in the fully fertile F_2 cross of these two species. Kühl (1938) afterwards published a further analysis showing that eight local wild species were all homozygous and hence stable, yet the interspecific crosses were fully fertile. Eleven genes for flower characters were recognized.

SPECIES HYBRIDIZATION

On the other hand, the analysis of the genus Zauschneria on the coast of California by Clausen, Keck and Hiesey (1940) shows that *Z. septentrionalis* in the north and *Z. cana* further south can be crossed readily, providing a fertile F_1 but the F_2 consists only of weak individuals. In both species $n = 15$. In nature, were these species sympatric, backcrosses would no doubt produce a hybrid swarm mainly of weak individuals, but particular combinations might be strong enough even to replace a parent species. Keck's description of the northern form as a new species is no doubt justified, as it has matted herbaceous (not woody) stems, broader (not filiform), subentire and densely canescent leaves.

The English primrose (*Primula vulgaris*) and oxlip (*P. elatior*) occur in the same woods in East Anglia and intercross, but each wood is isolated. Ecological preferences keep the species mainly in different parts of the wood (Valentine, 1949a). Reciprocal crosses were made, which gave a low yield of viable seeds. The vigor of the F_1 is variable, but when it is selfed or backcrossed a better yield of seeds is obtained. In this case ecological and genetic barriers prevent fusion of the species, the mixed populations appearing to be in equilibrium.

The genus Balsamorhiza, containing about a dozen species in the Western States, shows various conditions of intercrossing (Ownbey and Weber, 1943). *B. sagittata* and *B. Careyana* have slightly different habitat preferences, but at a point of contact intermediates (hybrids ?) were found. *B. Careyana* and

B. deltoidea on the contrary merge completely (with the formation of intermediates) where they meet. The extremes of the series are species, morphologically and ecologically, but biologically they are "parts of the same unit," and all attempts to separate them are futile. Although hybridization has obscured the discontinuity which perhaps existed originally, yet it has not submerged either species. Some of the other species do not meet and therefore do not intercross, yet members of two very distinct subgenera hybridize when in the same neighborhood, producing hybrid swarms with all the character recombinations. The authors reach the conclusion that "hybridization between widely separated entities may not indicate the desirability of a complete revision of time-tested taxonomic concepts so much as a need for better understanding of the nature and development of genetic barriers"... "the axiomatic application of hybrid sterility or inviability to the problem of species delimitation may be as unsatisfactory as the axiomatic application of the criterion of morphological difference." Evolutionary divergence between populations is a measure of the effectiveness of the barrier between them, not of its nature. Distinctions between geographic and genetic barriers may have no significance in evolution or in systematics.

We may next consider the tree genus Catalpa. *C. ovata* Don is native to China, *C. bignonioides* Walt. to North America. The reciprocal hybrids, known as × *C. hybrida*, are fertile, the F_1 showing hybrid vigor and the F_2 segregating. All have $2n = 40$ chromosomes. In the hybrids, as in the parents, the pollen meiosis is regular (Smith, 1941). These two species have been separated geographically for geological ages, yet they differ only by gene mutations and no taxonomist would unite them because of their interfertility. A fossil Catalpa occurs in the Oligocene of the Isle of Wight.

The careful study of hybridization in nature thus throws so much light on the question of species relationships that a few more specific cases may be cited, from the many in the literature. The paleontological aspects of species enters into a case in the oaks, described by Chaney (1949). *Quercus wizlizenii* A. DeCand., the interior live oak of California, hybridizes with *Q. kelloggii* Newberry, the California black oak, producing *Q. morebus* Kellogg, "a species restricted in distribution and numbers." The Tertiary equivalents of the parent species occur in the Miocene—Pliocene flora of Central California, and a third species, *Q. remingtoni* Condit, is probably the hybrid between them. The evidence indicates that hybridization was taking place on the western slopes of the Sierras several million years ago as it is now between the modern descendant species. Yet they have not merged, and *Q. remingtoni* is not a direct ancestor of *Q. morebus* but a Tertiary hybrid black oak.

Another instance where the "fourth dimension" (time) enters in is described by Mason (1949) in Pinus. There is evidence that *P. remorata* and *P. muricata* were distinct species in the Pleistocene of California. *P. remorata,* now confined to Santa Cruz island, is near extinction. *P. muricata* on the mainland occurs discontinuously in colonies along the opposite coast. The prevailing east winds carried the pollen of *P. remorata,* which thus introduced genes of this species into the southern range of *P. muricata.* The latter species has probably reached the island since, where the two species now form hybrid swarms. In this way *P. remorata,* which was formerly a "good" species, has been practically eliminated on the island.

Another case in which natural interspecific crossing has affected the phylogenetic development of both species is that of guayule (*Parthenium argentatum*) and mariola (*P. incanum*). These species cover much the same range in northern Mexico and western Texas. They mingle freely and there has been much introgression of genes (Anderson). From wild seeds collected, Rollins (1944) grew over 205,000 plants of guayule and nearly 1,000 of mariola. The natural hybrids numbered 162 in guayule, or less than 0.1 per cent., and in mariola only one plant. The trichomes of the two species are radically different, and this was used as an index of hybridity. The two species also differ in foliage and growth habit, and mariola has smaller heads and florets, the ray florets less deeply divided. The amount of bad pollen in each species varies from 5 per cent. to over 50 per cent. Both species show chromosome numbers which range in guayule from $2n = 36$, to 54, 58, 72, 74 and 108–112, and in mariola from 36 to 54, 72, 81, 90. Polyploidy and aneuploidy are both involved in this process of introgression. Some hybrids have very good pollen, others show the usual meiotic irregularities. From all the evidence, it is highly improbable that these two species evolved in separate areas and have since come together. Rather, evolution appears to have taken place, including polyploidy and finally apomicty, under conditions of continuous introgression.

A case with very interesting features is in the genus Solidago (Goodwin, 1937). *S. rugosa* Mill. and *S. sempervirens* L. are two very distinct species, and × *S. asperula* Desf. has long been recognized as the hybrid between them. *S. rugosa* inhabits open meadows and *S. sempervirens* salt-marshes, having the characteristic fleshiness of halotypes such as *Aster tripolium.* Plants were collected and inbred in the greenhouse for two generations, both species being constant. Reciprocal crosses were made, both species having $n = 9$ chromosomes. Meiosis was normal in F_1, F_2 and backcrosses, and there was no aberrant pollen sterility. The differences between these species are not only in habitat, but also in the stolons, leaves, pubescence, inflorescence and various characters of the flowers and heads. Goodwin found six inherited differences in the leaves alone, and one may estimate at least 28 character differences. As these frequently depend on multiple quantitative genes, the number of interspecific genes must be much greater. The presence of ecological adaptation as well as marked morphological differences between two species which are nevertheless fully fertile shows how futile it would be to use interfertility as a better criterion of species than morphological difference.

A pair of species in Lactuca (Whitaker, 1944) are of interest because "These two very distinct species are for the most part dependent upon simple gene differences." *L. graminifolia* and *L. canadensis* were crossed, the F_1 and later generations being as fertile as either parent species, their meiosis normal. The former species occurs from South Carolina to Florida and Texas, the latter from Nova Scotia to Saskatchewan and south to Arkansas, Louisiana and Georgia. Natural hybrids are found in South Carolina where their very different ranges overlap. Both species were uniform in cultures and reciprocal crosses were made. The F_1 is more or less intermediate, but the annual habit of *graminifolia* is dominant to the biennial habit of *L. canadensis.* The grey pollen (versus orange) and purple blue (versus orange yellow) ligules of *L. graminifolia* were dominant, the pollen color segregating very sharply as a sporophytic character. The lobed, pinnatifid (versus entire, lanceolate) leaves of *L. canadensis,* were dominant, but modifying factors determined the nature of the lobing. *L. canadensis* is much taller but with shorter involucral bracts, the F_1 being intermediate in these respects. In other characters the differences were more complex. This is a good example of a pair of species with four or five very marked differences and probably a large number of minor ones. There is very little hybridization, because *L. graminifolia* flowers earlier than *L. canadensis.* The hybrids are also regarded as unsuccessful because of "their inability to encounter an ecological niche to which they are well adapted." These two species have n = 17 chromosomes and they probably diverged from a common amphidiploid ancestor produced by a cross between species having 9 and 8 chromosomes respectively. It would be ridiculous to combine these species or reduce one to a subspecies because their hybrids are fertile.

By way of comparison, a couple of recent cases from animals may be cited. The butterflies, *Basilarchia arthemis* and *B. astyanax* intercross on the line where they meet, in a zone from New England to Pennsylvania, northern New York, central Michigan, Wisconsin, and Minnesota (Hovanitz, 1949). The hybrids produced go under the name *B. proserpina.* This state of affairs was known in 1879 and is the same today, but the species do not merge. Hovanitz cites similar conditions in *Colias philodice* and *C. eurythema,* which overlap in much of their area and intercross, so that some 10 per cent. of the population may be intermediate hybrids. The F_2 swarm are multifactorial and backcrosses are fertile, but the species are not obliterated. This is partly because they have different food plants and there is a high mortality of the hybrids on the wrong food.

According to Shrode and Lush (1947), zebu cattle (*Bos indicus*) are no more sterile in crosses with European cattle (*Bos taurus*) than are the corresponding intraspecific crosses. "They differ widely in many kinds of characteristics, including things as different as horns, skull shape, dewlap, voice, size of digestive tract, resistance to some tropical diseases, and mental traits." They also differ in the presence of the zebu shoulder hump, not to mention the marked differences in coat color. Yet the authors suggest that because these two species, which must have evolved and become domesticated in widely separate areas (one tropical and the other temperate) are interfertile, "probably this should not be called a species cross." I submit that to merge them when morphology and physiology speak so loudly to the contrary is a misuse of the fact of interfertility.

The recognition and naming of plant and animal kinds must have existed from the time man began to speak. The categories of taxonomy have become progressively more exact since that time. Modern taxonomy fulfils this function of classification as well as the intricacies of evolutionary processes will permit. With the recognition of evolution, taxonomy acquired a second function, the building up of phylogenetic lines on the basis of evolutionary theory. Neither of these processes can ever be complete, but every taxonomist recognizes that there are clearly defined species and species which the taxonomist must define by "drawing a line somewhere." The latter cases must always lead to differences of opinion, but it is clear from the modern genetical and cytological work that mere divergence does not necessarily produce intersterility. It is equally clear that intersterility can arise in the absence of phenotypic difference. Since the whole function of taxonomy is based on the recognition and discrimination of phenotypes, the taxonomist must always have the last word in determining what is a species. In taxonomic work there is no rule-of-thumb criterion, such as sterility, which will take the place of wide experience and careful judgment.

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GENETIC TUMORS IN NICOTIANA

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INTRODUCTION

Most plant tumors originate directly as the result of a chain of reactions started by some environmental tumor-inciting agent. Tumors in this category include those stimulated by bacteria, viruses, insects, nematodes, selected chemicals including plant growth regulators, and other factors of external origin which are not generally passed on in the progeny of the plant. Such tumors are never spontaneously initiated from within the plant but are always dependent on factors external to the plant for their inception.

Genetic tumors, on the other hand, arise spontaneously, are initiated solely by factors within the plant cells, and always occur in entire populations of plants of a particular genotype as, for example, in certain interspecific hybrids of Nicotiana. In many respects these plant tumors are the counterpart to cancer in animals, notably, both occur spontaneously and the incidence of both is highest among organisms that have passed maturity. Evidence is presented in this study which is indicative that these tumors are controlled by genes affecting the growth regulatory mechanism and appear when the system is thrown out of balance in plants of certain genetic constitution.

EXPERIMENTAL RESULTS

In taking field notes on a total of more than sixty different interspecific hybrids of Nicotiana it was observed that certain ones consistently developed tumors. Some of these tumorous growths were strikingly large, up to 50 mm. in diameter, and occurred on almost all parts of the plant (fig. 2). Wounding did not encourage tumor formation if the terminal growing point was not injured. When the terminal buds were removed from actively growing plants, tumors appeared within ten days, while plants that were wounded by cutting halfway through the stem continued growing normally.

The tumors in most instances were not fatal to the plant and usually appeared after flowering was well advanced and terminal meristematic growth had become less active. No tumors were found as long as the plants were in an actively growing condition, an observation that was also made by other workers (Whitaker, 1935, Kostoff, 1943). The stems adjacent to the tumors never produced additional flowers, but commonly new shoots started from the basal parts of the plant and these eventually bore flowers (fig. 4). In some hybrids the tumors appeared at an early age when the plant had only one or two true leaves (figs. 1 and 9). At this stage the plants were not actively elongating.

TABLE 1

INTERSPECIFIC HYBRIDS OF NICOTIANA WHICH WERE PREVIOUSLY REPORTED TO FORM GENETICALLY CONTROLLED TUMORS

Female parent	Somatic chromosome number	Male parent	Somatic chromosome number	Worker
N. glauca	24	*N. langsdorffii*	18	Kostoff 1943
N. glauca	24	*N. longiflora*	20	Kostoff 1943
N. glauca	24	*N. plumbaginifolia*	20	Kostoff 1943
N. langsdorffii	18	*N. glauca*	24	Kostoff 1943
N. miersii	24	*N. langsdorffii*	18	Kostoff 1943
N. paniculata	24	*N. langsdorffii*	18	Kostoff 1943
N. paniculata	24	*N. sanderae*	18	Kostoff 1943
N. quadrivalvis	48	*N. sanderae*	18	Whitaker 1934
N. rustica	48	*N. cavanillesii*	24	Kostoff 1943
N. rustica	48	*N. sanderae*	18	Kostoff 1943
N. rustica	48	*N. alata*	18	Kostoff 1943
N. rustica	48	*N. langsdorffii*	18	Kostoff 1943
N. rustica var. *humilis*	48	*N. tabacum* var. *sanguinea*	48	Kostoff 1930
N. suaveolens	32	*N. langsdorffii*	18	Kostoff 1930
N. tabacum var. *wigandioides*	48	*N. sanderae*	18	Kostoff 1930
N. tabacum var. *sanguinea*	48	*N. alata*	18	Kostoff 1930

In a few hybrids, tumors were found only on the roots of a part of the population. These tumors were identical in appearance to those found on the roots of plants with above-ground tumors and also resembled those caused by crown gall bacteria (fig. 11). However, all attempts to isolate causative pathogens from tissue plantings taken from various portions of these root tumors and grown on nutrient agar were unsuccessful. In one case a plant with root tumors later developed characteristic stem tumors when it was transplanted from the field to the greenhouse. It would appear, therefore, that the root tumors are early manifestations of the physiologic condition that later produces typical stem tumors, but that the above ground parts of the plant do not develop typical stem tumors as long as the plant is actively growing or has not reached the point of physiological maturity required for

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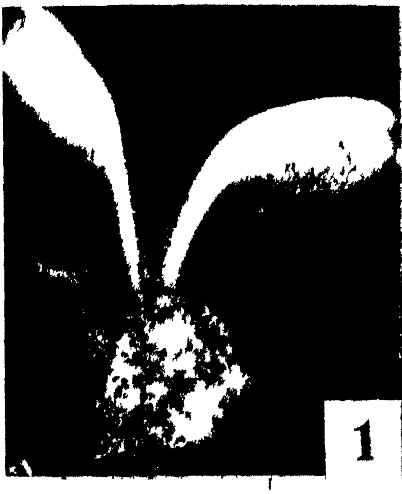
FIGURE 1. Tumors on a month-old seedling of the F_1 interspecific hybrid, *N. glauca* × *N. langsdorffii*, after colchicine treatment. Such seedlings later recover and grow normally until the late flowering period when they again form tumors. (× 3)

FIGURE 2. Close view of tumors on the F_1 hybrid of *N. glauca* × *N. langsdorffii*, showing nature of tumorous growths. (× ½)

FIGURE 3. Plant with triple genome combination, *N. debneyi-glauca* × *N. langsdorffii*, with characteristic tumorous growths. This plant had only *N. debneyi* cytoplasm, but readily formed tumors soon after blooming. (× ⅒).

FIGURE 4. Amphidiploid, *N. glauca-langsdorffii*, with tumor formation subsequent to flowering. No new terminal growth occurred adjacent to the tumorous tissue, but arose as shown from the basal parts of the plant and produced normal flowers. (× ⅒)

FIGURE 5. Dying plant of *N. debneyi-glauca* × *N. langsdorffii* with abnormal tissue on the stems and roots. There is a mass of decomposing tumor tissue at the base of the stem. (× ⅕).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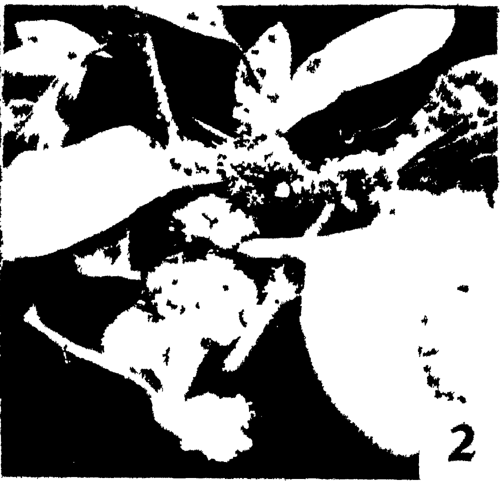
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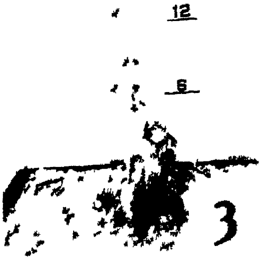

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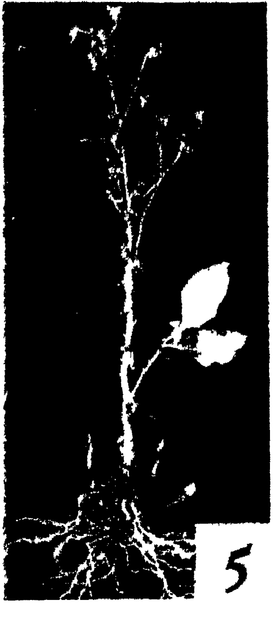
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tumor development. This interpretation is supported by the fact that several hybrids that Kostoff (1943) described as forming stem tumors, in this study formed only root tumors (e.g. *N. glauca* × *N. plumbaginifolia*).

In this study tumors were found in nineteen interspecific combinations as shown in table 2, sixteen of which have never before been reported.

TABLE 2

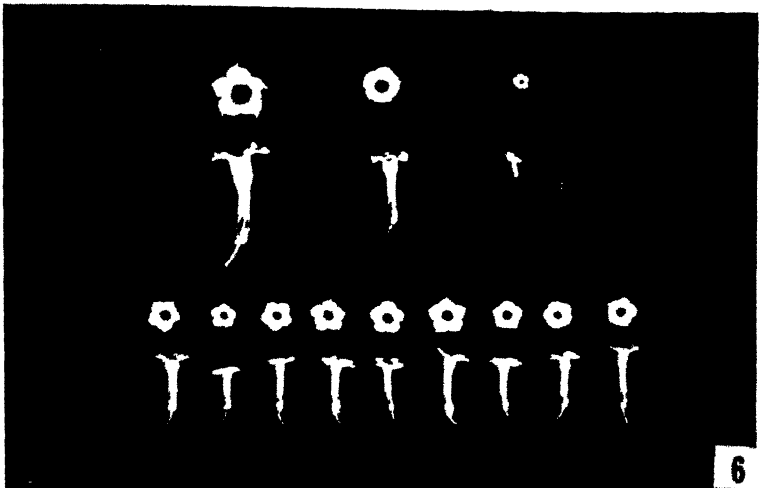
INTERSPECIFIC GENOME COMBINATIONS OF NICOTIANA IN WHICH GENETICALLY-CONTROLLED TUMORS WERE FOUND IN THIS STUDY

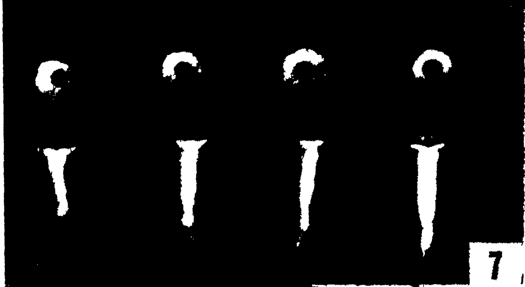
Female parent	Somatic chromosome number	Male parent	Somatic chromosome number	Part of plant affected
**N. debneyi*	48	*N. plumbaginifolia*	20	Roots only
**N. debneyi-glauca*	72	*N. alata*	18	Roots only
**N. debneyi-glauca*	72	*N. langsdorffii*	18	All parts
**N. debneyi-glauca*	72	*N. plumbaginifolia*	20	Roots only
**N. debneyi-glauca*	72	*N. sanderae*	18	Roots only (1 plant all parts)
**N. debneyi-plumbaginifolia*	68	*N. glauca-langsdorffii*	42	All parts
**N. debneyi-tabacum*	96	*N. alata*	18	Roots only
**N. debneyi-tabacum*	96	*N. langsdorffii*	18	All parts
**N. debneyi-tabacum*	96	*N. plumbaginifolia*	20	Roots only
**N. debneyi-tabacum*	96	*N. sanderae*	18	Roots only
**N. debneyi-tabacum* × *N. debneyi*	72	*N. debneyi-tabacum* × *N. debneyi*	72	All parts (1 plant in pop. of 12 plants)
N. glauca	24	*N. langsdorffii*	18	All parts
N. glauca	24	*N. plumbaginifolia*	20	Roots only
**N. glutinosa-glauca* × *N. glauca*	36	*N. glutinosa-glauca* × *N. glauca*	36	All parts (1 plant in pop. of four)
N. langsdorffii	18	*N. glauca*	24	All parts
**N. plumbaginifolia*	20	*N. glauca*	24	Roots only
**N. suaveolens*	32	*N. sanderae*	18	Roots only (a few plants formed top tumors)
**N. tabacum-glauca*	72	*N. alata*	18	Roots only
**N. tabacum-glauca*	72	*N. sanderae*	18	Roots only

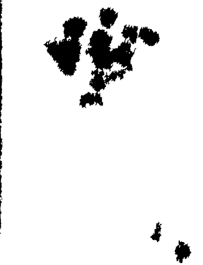
*Not previously reported.

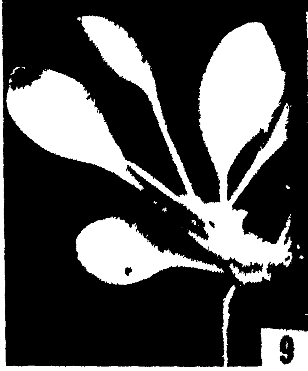
POSSIBLE EXPLANATIONS OF THE CAUSATIVE AGENT

Three theories that have been advanced to explain the cause of genetically controlled tumors are: (1) immunological relationships, (2) heterogeneity of tissues and abnormal mitoses, and (3) cytoplasmic disturbances. The writer proposes a fourth theory based upon abnormal phytohormone relationships. It is necessary to rule out a virus, bacterium, or other pathogenic entity as a causative agent since all attempts over a period of years and by many different workers have failed to isolate an environmental causative agent from the genetic tumors on Nicotiana hybrids tested. In like manner, attempts to transmit an entity by grafting experiments from tumor-forming plants to normal plants have failed. Seedlings grown in the greenhouse on









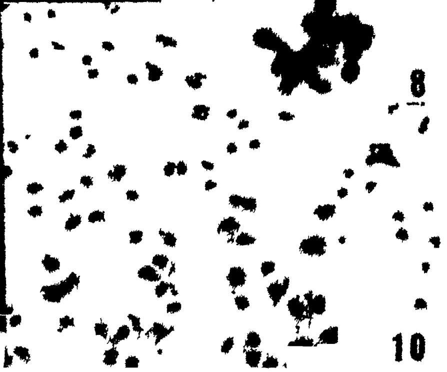


FIGURE 6. Corollas of plants combining genomes of *N. debneyi* and *N. tabacum.* Top row, left to right—*N. tabacum*, amphidiploid *N. debneyi-tabacum*, *N. debneyi.* Bottom row, plants with two genomes of *N. debneyi* plus random segregating chromosomes of *N. tabacum*, (× ¼).

FIGURE 7. Corollas of plants combining genomes of *N. glauca* and *N. langsdorffii.* Right to left, LL (diploid *N. langsdorffii*), GLL, GGLL (amphidiploid *N. glauca-langsdorffii*) and GGGL, (× ⅓).

FIGURE 8. Photomicrograph of cell of the amphidiploid, *N. glauca-langsdorffii* at Anaphase I showing delayed separation of one bivalent. A few cells exhibit even more pseudo bridge—like structures, (× 3500).

FIGURE 9. Seedling of the amphidiploid *N. glauca-plumbaginifolia* showing tumors, (× ⅚).

FIGURE 10. Photomicrograph of pollen mother cells of the amphidiploid *N. glauca-*

steam sterilized soil readily formed tumors in the seedling stage indicating that the tumor causing factor must come with the seed. All available evidence to date indicates that the tumors described on Nicotiana hybrids are not the result of an environmental tumefacient agent and must be considered of genetic origin.

Immunological relationships: The theory of an immunological reaction as an explanation of tumor formation was first proposed in 1930 by Kostoff. In a later publication Kostoff (1943) did not mention the immunological reaction theory but proposed an entirely different explanation for tumors in the cross *N. glauca* × *N. langsdorffii*, thus indicating that the immunological reaction theory had been discarded by its own author.

There are several phenomena that cannot be explained by this theory. *N. langsdorffii* can be used either as stock or scion in compatible grafts with *N. glauca*, and there is no evidence that tumor-causing substances diffuse across the graft to cause tumors in *N. glauca*. It is likewise difficult to explain by this hypothesis why tumors appear only when meristematic elongation is retarded, i.e., in the seedling stage and late blooming stage. In addition there are no experimental results which completely support the immunological reaction theory in Nicotiana hybrids other than that given by Kostoff (1933) which even he cautioned were only indicative. These data consisted of results of a precipitin reaction between the extracts of the paternal and maternal species put together *in vitro*. He did not give the method used and it is, therefore, impossible to repeat the experiment exactly. However, there has been some doubt in recent years concerning the validity of the precipitin test itself when applied to plant extracts exclusively.

Heterogeneity of tissues and abnormal mitoses: Kostoff (1943), in an attempt to explain the cause of tumors in the cross *N. glauca* × *N. langsdorffii*, proposed that the stimulating factor for cell proliferation was most likely the "heterogeneity and mosaicism" of the plant tissues, "recalling the phenomenon of hybrid vigor." He explained that the mosaic tissue arose from "highly disbalanced" cells of the growing tip which in turn were the result of abnormal mitoses, and that in the apical promeristem there are somatic exchanges of segments between chromosomes of *N. glauca* and *N. langsdorffii* which increase in numbers as the plant becomes older. Quoting from Kostoff (p. 618), "Such genetically different cells, together with the normal ones that have remained, divide with various cell division rates and form tumorous instead of differentiated organs." Whitaker (1934) found no significant number of chromosomal irregularities in mitotic divisions of tumor cells that he examined, evidence which does not support this theory of Kostoff.

If there were abnormal mitoses in the cells of the apical promeristem, it would be logical to expect that meiosis would likewise be abnormal in the pollen mother cells differentiated from these same promeristem cells. Meiosis was studied in over 200 pollen mother cells from colchicine-induced amphidiploid plants which later formed tumors. In general all stages of reduction division were normal (fig. 10), and the chromosome numbers were constant in all cells where chromosomes were counted. In less than one per cent. of the Anaphase I figures there was delayed separation of one of the bivalents, while in still fewer figures two or more bivalents seemed to be connected with chromatic strands, which on casual observation might be mistaken for bridges resulting from dicentric chromosomes. In figure 8 there were 20 chromosomes at each pole while the remaining pair of chromosomes seemed to be moving tardily to respective poles. In no cases were chromosome fragments observed remaining in the cytoplasm between the two poles at Anaphases I or II, as would be expected were the above condition a true chromosomal bridge. Further evidence of meiotic regularity was afforded by the uniformity of progeny of five lines of amphidiploids through two generations of selfing. Additional data in table 3 show that the five lines of amphidiploids and two backcrosses had a reasonably high amount of stainable pollen grains.

TABLE 3

STAINABLE POLLEN IN *Nicotiana glauca* × *N. langsdorffii* HYBRID COMBINATIONS

Line	Genomes involved	Designation	Size of pollen grains	% Stainable pollen
50191	*GGLL	Amphidiploid	20–25 u	78.7
50192	GGLL	Amphidiploid	17–25 u	94.8
50194	GLL	Backcross to *N. langsdorffii*	18–25 u	70.6
50195	GGLL	Amphidiploid	20–26 u	90.5
50196	GLL	Backcross to *N. langsdorffii*	18–25 u	77.8
50197	GGLL	Amphidiploid	18–24 u	88.5
50198	GGLL	Amphidiploid	23–25 u	92.8
50211	LL	*N. langsdorffii*	18–25 u	88.3

*G = one *N. glauca* genome.
*L = one *N. langsdorffii* genome

Kostoff reported that his amphidiploid, *Nicotiana glauca-langsdorffii* was not true-breeding, having as high as 55% deviates in the F_3, and only 51% stainable pollen (Kostoff, 1939). It is conceivable that he did not have a true amphidiploid though it is impossible to ascertain its origin. In three different publications he gives as many methods for producing the amphidiploid. In 1935 it was produced by crossing the triploid, (*N. glauca* × *N. langsdorffii*) × *N. langsdorffii* with *N. glauca* (Kostoff, 1935). In 1939 it was produced parthenogenetically by crossing the F_1 hybrid, *N. glauca* × *N. langsdorffii* with *N. langsdorffii*, and named *N. vavilovii* (Kostoff, 1939). In 1943 the origin is still obscure for he says "I doubled the chromosomes in the F_1 hybrids and produced self-fertile allopolyploids *N. glauca* × *N. langsdorffii* shortly called *N. vavilovii* (1935, 1938) which formed tumors like F_1 hybrids and represented very convenient objects for tumor investigations" (Kostoff, 1943, page 616). If this amphidiploid did not represent the two complete genomes from each parental species, it would explain not only why he found wide segregation in the progeny but also abnormal meiotic behavior. It does not, however, fully explain the abnormal mitotic anaphases he reported in the root tips. This latter condition might well be only a step in a chain of reactions which ultimately cause tumor formations as a plant response to an unbalanced phytohormone mechanism.

The theory based on abnormal mitoses is also inadequate to explain why tumors appear in the early seedling stage before there has been opportunity for a build-up in the number of highly disbalanced cells.

Cytoplasmic disharmonies: Whitaker (1934) proposed that there was a cytoplasmic disturbance caused by the introduction of the chromosome complement of *N. langsdorffii* as a male into the cytoplasm of *N. glauca.* This was based in part upon his unconfirmed observation that the reciprocal cross did not produce tumors. The writer found tumors on hybrids of the reciprocal cross that were in all respects identical in appearance and development to those in the F_1 *N. glauca* (female) x *N. langsdorffii* (male). Similar observations made in the cross *N. langsdorffii* (female) x *N. glauca* (male) by Kostoff (1943) and Dr. H. H. Smith, Cornell University (unpublished), clearly establish the fact that the tumors found in both crosses are identical.

There is some additional evidence which makes even more unlikely Whitaker's cytoplasmic explanation. Tumors were formed in all parts of the plant produced by the cross of the amphidiploid *N. debneyi-glauca* x *N. langsdorffii* (figs. 3 and 5). The amphidiploid was produced by doubling the chromosome set of the interspecific hybrid of *N. debneyi* x *N. glauca,* so that the amphidiploid contained only the cytoplasm of *N. debneyi.* Yet when the chromosome complement of *N. langsdorffii* was introduced into this plant which had no *N. glauca* cytoplasm, tumors were formed. The cytoplasmic theory is, therefore, not supported by this later evidence and the writer knows of no subsequent experimental data which support it. Not only would it be difficult to explain tumor formation in all the hybrid combinations by this theory, but it would also be hard to reconcile the cytoplasmic theory with the established fact that tumors appear only when the plant is not growing rapidly.

Abnormal phytohormone relationships: The above three theories fail to explain completely the cause of spontaneous tumors found in the hybrids listed in tables 1 and 2. Consequently, the following five statements are marshalled in support of a more comprehensive theory of spontaneous tumor formation. All five statements are reasonably well substantiated by experimental data.

(1) Phytohormone metabolism in plants is controlled by gene action.

(2) *N. langsdorffii* possesses a genetically-controlled ability to make phytohormones less effective.

(3) Phytohormones may produce tumors which are similar in many respects to those produced by chemicals, bacteria, viruses, and other environmental agents.

(4) *N. langsdorffii* is more effective in tumor-forming hybrids than any other species of Nicotiana tested.

(5) Plants produce tumors principally during a period of reduced terminal meristematic growth.

The above five statements will be discussed in the order given.

Statement one: Different species and varieties of plants have been shown to have heritable variations in their metabolism of auxin. Dwarf "nana" corn differs from normal-sized corn by a single gene, but is dwarfed in growth because there is less auxin available in its tissues for normal cell elongation (Van Overbeek, 1935). Hybrids of Epilobium have an auxin production directly proportional to the size of the plant (Hinderer, 1936). The metabolic process affecting auxin production is the determining factor for the growth of the pea stem (*Pisum sativum*), the tall pea varieties having more available auxin than the dwarf forms (DeHaan and Gorter, 1936). These facts strongly indicate that inactivation or lack of function of phytohormones is controlled by gene action.

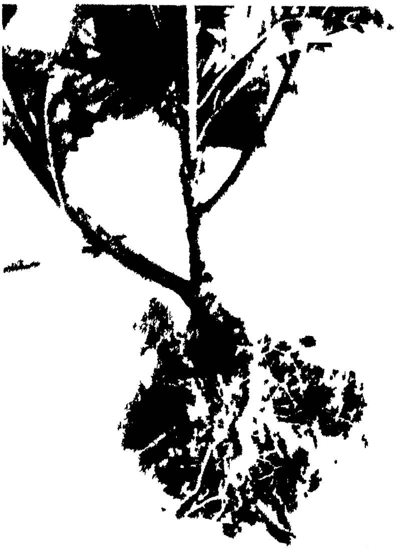


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FIGURE 11. Mass of root tumors on a plant of *N. debneyi-glauca* × *sanderae*. All indications are that these tumors, found in about 20 per cent. of the population, are early manifestations of the condition that later causes stem tumors, (× ⅓).

Statement two: Studies of the morphogenetic differences between *Nicotiana alata* and *N. langsdorffii* show that the corollas follow the same general growth pattern but differ in cell elongation (Nagel, 1939). Corollas of *N. langsdorffii* are smaller than those of *N. alata* chiefly because the cells are less elongated. There is evidence that growth regulators are inactivated in the corollas of *N. langsdorffii*. Equal concentrations of indoleacetic acid applied to the corollas of both species result in further cell elongation of the *N. alata* corolla, but there is little or no response in that of *N. langsdorffii.* These experiments seem to indicate that the principal difference between the two species lies in the ability of each to utilize the growth regulator and that phytohormones are inactivated or do not operate effectively in *N. langsdorffii.* This characteristic is probably genetically controlled.

Statement three: Roots, stems, and buds all behave in a comparable way in that growth is inhibited by relatively high concentrations of auxins (Thimann, 1937). Furthermore, when indole-acetic acid is used at concentrations that inhibit growth, galls similar to those produced by crown gall bacteria are formed on a series of plants including *Nicotiana tabacum.* (Brown and Gardner, 1936; Friedman and Francis, 1942). It is a significant fact that wound phytohormones have been reported to cause spontaneous tumors in decapitated bean plants (Flint and Moreland, 1945). Thus it has been demonstrated frequently that relatively high concentrations of phytohormones produce tumors in plants, and under some conditions tumors have been reported to result from growth regulators generated within the plants.

Statement four: In an analysis of the sixteen tumor-forming hybrids listed in table 1, seven contain *N. langsdorffii* as one parent. Similarly, five of the ninteeen hybrids found by the writer to produce tumors contain *N. langsdorffii* as one parent. A further analysis of the latter nineteen hybrids reveals that in those which produced tumors on all parts of the plant, all had *N. langsdorffii* as a parent, while the eleven hybrids which did not involve *N. langsdorffii* as a parent produced only root tumors, with the exception of three plants. It thus appears that *N. langsdorffii* is a more effective component in tumor-forming hybrid combinations than any other species of Nicotiana tested.

The tumor-forming ability of the *N. langsdorffii* genome remains effective even when overbalanced by additional genomes from *N. glauca.* Plants have been obtained with as many as three genomes of *N. glauca* to one of *N. langsdorffii,* (fig. 7). In all plants shown in table 4 characteristic tumors formed which were identical in all respects to those already described. Although the plants flowered successively later as the ratio of *N. glauca* genomes was increased, the tumors always appeared about four weeks after the first flower opened.

Additional evidence on the nature of the cause of tumors was found in two plants segregating for chromosomes of *N. tabacum* and *N. glutinosa* as shown in table 2. Both of these plants died soon after the appearance of the tumors.

The first was a plant out of a population of 12 segregants resulting from selfing the plant, *N. debneyi-tabacum* × *N. debneyi.* All 12 plants more nearly approached the appearance and growth of *N. debneyi* than the backcross parent, but had varying degrees of *N. tabacum* characteristics (fig. 6). This would be expected as a result of the segregation of *N. tabacum* chromosomes. No tumors were noted either in the original amphidiploid, *N. debneyi-tabacum,* nor in the first backcross generation, *N. debneyi-tabacum* × *N. debneyi,* nor in 11 of the above described segregants. Apparently the

TABLE 4

TUMOR-FORMING INTERSPECIFIC HYBRIDS BETWEEN *N. langsdorffii* AND *N. glauca* WITH VARYING GENOMES OF EACH SPECIES

Genome combination	How produced	Number of genomes of *N. langsdorffii*	Number of genomes of *N. glauca*	Ratio of genomes of *N. langsdorffii* to *N. glauca*	Number of chromosomes of *N. langsdorffii*	Number of chromosomes of *N. glauca*	Ratio of *N. langsdorffii* chromosome to *N. glauca* chromosomes
GL*	*N. langsdorffii* × *N. glauca* and reciprocal	1	1	1:1	9	12	3:4
GLL	Amphidiploid *N. glauca-langsdorffii* × *N. langsdorffii*	2	1	2:1	18	12	3:2
GGL	Amphidiploid *N. glauca-langsdorffii* × *N. glauca*	1	2	1:2	9	24	3:8
GGLL	Colchicine doubling of the F_1 interspecific hybrid	2	2	1:1	18	24	3:4
GGGL	Amphidiploid *N. glauca-langsdorffii* × 4N *N. glauca*	1	3	1:3	9	36	1:4

*G = *N. glauca* genome.
L = *N. langsdorffii* genome.

metabolism in these latter plants was such that the phytohormone relationship was not disturbed to the extent that tumors were initiated. However, the fact that tumors did occur in the one plant among the segregating population of twelve indicates that the ability for balanced cell division and tissue correlation was disrupted by the loss of certain *N. tabacum* chromosomes. As previously mentioned, gene action was found to regulate auxin concentration in dwarf nana corn (Van Overbeek, 1935) and undoubtedly phytohormone metabolism is likewise gene-controlled in *N. tabacum.* Thus, when the chromosome carrying this gene or genes is lost, control of the phytohormone regulatory mechanism would also be lost. The ultimate result would be a disturbance in phytohormone balance. Whether or not this is a correct explanation, the fact remains that tumor formation in the one plant described was associated with the loss of certain unidentified chromosomes of *N. tabacum.*

The second plant was one of four segregants in a population resulting from the selfing of *N. glutinosa-glauca* × *N. glauca,* in which case it was the *N. glutinosa* chromosomes that were lost. This plant formed extremely large tumors, especially at the base of the stem.

Statement five: Another pertinent fact is that tumors appeared in the *Nicotiana* hybrids only during the stage of plant development when terminal meristematic growth in the stem was reduced. When the terminal buds were removed from actively growing plants, tumors readily formed at the tip of the stem and developed in other parts of the plant within 10 days, at least four weeks sooner than if the plant were allowed to grow normally. This was interpreted to indicate that there was a changed concentration of phytohormones when the apical cells were no longer actively elongating, and this altered concentration of phytohormones might be the condition initiating tumor formation.

All the above statements, considered as a whole, support the hypothesis that in certain genome or chromosome combinations of Nicotiana species, particularly those involving *N. langsdorffii,* the genetically-controlled phytohormone metabolism, which in the species themselves produces normal growth, is disturbed. This disturbance in the growth regulatory balance stimulates certain tissues of the hybrids to become meristematic resulting in the formation of undifferentiated masses of plant material, generally referred to as tumors.

SUMMARY

Studies were made of thirty-one interspecific tumor-forming hybrid combinations in Nicotiana, sixteen of which have not been previously reported. In these studies little evidence was found supporting three theories proposed prior to this study to explain the cause of genetic tumors. Such tumors are always spontaneously initiated from within the plant, are produced in entire populations of certain crosses, are transmitted to the progeny by factors carried with the seed, and occur only when the plant is not growing rapidly. Of all the species tested, *N. langsdorffii* was involved in more tumor-forming combinations than any other single species. In two

interspecific combinations tumor formation was associated with loss of chromosomes. Certain experimental data which cannot be explained by existing theories indicate that genetic tumors may have their origin in abnormal phytohormone relationships.

Species comprise harmonious systems in which developmental processes, including phytohormone utilization, are integrated to produce normal growth. However, in certain interspecific combinations of Nicotiana, particularly with *N. langsdorffii* as one parent, there is a disturbance of this growth regulatory mechanism. It is hypothesized that this results, at maturity and occasionally in the seedling stage, in the inability of the plant to normally metabolize phytohormones which in turn, results ultimately in a stimulation of certain tissues to become meristematic and to grow abnormally without definite cell differentiation.

ACKNOWLEDGEMENT

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HYBRIDIZATION IN THE ANNUAL SUNFLOWERS: HELIANTHUS ANNUUS × H. ARGOPHYLLUS[1]

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Helianthus annuus, the common sunflower, hybridizes freely with other annual sunflowers. Hybridization of *H. annuus* with *H. petiolaris, H. Bolanderi* and *H. debilis* var. *cucumerifolius* has been described previously, (Heiser, 1947, 1949, 1950), and in the present paper natural and artificial hybrids of *H. annuus* × *H. argophyllus* are analyzed.

Helianthus argophyllus[2] occurs today in both Texas and Florida. The species was originally described by Torrey and Gray in 1842 from material collected in Texas, and the earliest collection which I have seen from Florida is dated 1893 (Rolfs, University of Florida). It has been known in cultivation as the "silver leaf sunflower" since 1889 (Burpee Seed Catalogue). In all probability it was originally native to Texas but has escaped from cultivation to become naturalized in Florida. In Texas it is found only in or near the southern coast (fig. 1) in regions of sandy soil. This species is one of the slowest growing and latest blooming of the annual sunflowers, generally coming into flower in late August or early September.

The common sunflower, *H. annuus,* is today widespread throughout much of North America and is not uncommon in southern Texas (fig. 1). This species seldom or never occurs on sandy soil and generally comes into bloom in late June or July. The differences between these two species in soil preference and in time of blooming are strong barriers to their hybridization. However, the two species do come into contact in nature and there is an overlap in blooming seasons since *H. annuus* does flower into September.

The two species are closely related judging from their morphology. The most conspicuous difference is found in the pubescence—the leaves, stems, and bracts of *H. argophyllus* are covered with long silky hairs giving the plant a silvery gray color, whereas the pubescence in *H. annuus* consists of short, rough hairs and the plant is green in color. The leaf in *H. annuus* is always serrate, and in *H. argophyllus* it is nearly entire or shallowly

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[1] This project has been aided by grants from the Penrose Fund of the American Philosophical Society and the Graduate School of Indiana University. I should also like to extend my thanks to the curators of the following herbaria—Gray Herbarium of Harvard University, University of Florida, Missouri Botanical Garden, New York Botanical Garden, Southern Methodist University, University of Texas, Texas A. & M. College, and the United States National Herbarium—for making their specimens of the annual sunflowers available for study.

[2] Watson (1929) lists the original collection of *H. tephrodes* Gray in synonomy under *H. argophyllus.* I have seen the type specimen of *H. tephrodes* and it is clearly not *H. argophyllus.* The specimen, moreover, comes from California which is outside of the range of *H. argophyllus. Helianthus tephrodes* has been referred to *H. niveus* (Benth.) Brandegee by Blake (1918, p. 187).

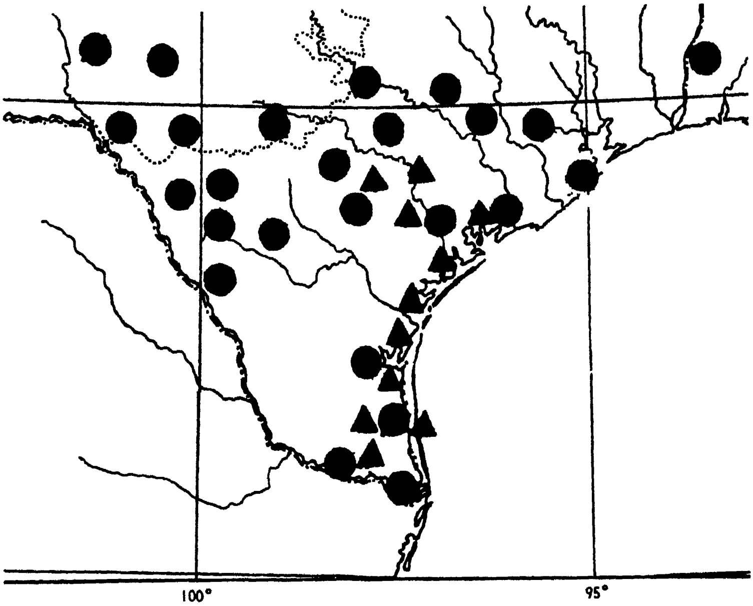

FIGURE 1. Map showing the distribution of *Helianthus annuus* (dots) and *H. argophyllus* (triangles) in southern Texas.

serrate. The achene in *H. annuus* is plump, in *H. argophyllus* it is rather flat.

NATURAL HYBRIDS

Putative natural hybrids between the two species have been collected in several localities in Texas by Dr. B. C. Tharp and by the writer (table 1). The specimens collected by Dr. Tharp are deposited in the herbarium of the University of Texas, and those of the writer are at Indiana University. The plants which are considered hybrids are intermediate between the parent species in pubescence, and match very closely the artificial hybrids which have been grown. In addition, the pollen fertilities of the putative natural hybrids show the same general range as those of the artificial hybrids (see below).

TABLE 1

PUTATIVE NATURAL HYBRIDS BETWEEN *H. annuus* AND *H. argophyllus* IN TEXAS

Collector and number	Locality	Pollen fertility
Tharp 1417	Kenedy Co.	33 per cent
Tharp 1639	Calhoun Co.	10 per cent
Tharp 48385	Kenedy Co.	25 per cent
Tharp 48385A	Kenedy Co.	17 per cent
Heiser 3072	Wilson Co.	25, 33, 40 and 42 per cent

The four hybrid plants collected by the writer along a roadside in Wilson County on June 17, 1949, were just coming into flower. Plants of *H. annuus* growing with the hybrids were in full bloom. In a field nearby where *H. argophyllus* grew in abundance a few of these plants were in bud although none was in flower on this date.

Neither at this locality nor at any other locality visited in eastern Texas were plants observed which appeared to be backcrosses. Either natural backcrosses are rare, or so similar to the recurrent parent in appearance that they are not readily detected in the field.

SPONTANEOUS AND ARTIFICIAL GARDEN HYBRIDS

History. Satsyperov (1916) reported on the cross of *H. annuus* x *H. argophyllus.* Using a cultivated form of *H. annuus* as the female parent he obtained seed for an F_1 generation, but he did not discuss the relative fertility of the hybrids. Cockerell (1916) called attention to the slow growth of *H. argophyllus* and mentioned that this "peculiarity is dominant in a cross with *H. annuus* x *lenticularis* (sic)." In 1918 he reproduced a photograph of a second generation cross between a red-rayed *H. annuus* and an *H. argophyllus.* In 1929 he again discussed this hybrid and stated that he found one hybrid with "about half of the pollen grains normal," and another with "hardly 10 per cent" normal pollen. Blakeslee (1945, p. 571) has also produced the hybrid between these two species.

F_1 hybrids. (4817). Two plants of *H. argophyllus* (seed from a herbarium specimen in the University of California, Cory 51242, San Patricio Co., Texas) were grown in an experimental field at Davis, Calif., along with plants of *H. annuus.* Open pollinated seed from *H. argophyllus* were planted the next year, and five of the eight plants secured were intermediate morphologically between *H. annuus* and *H. argophyllus.* The pollen fertilities of these five plants ranged from 10 to 33 per cent with a mean of 22 per cent. Upon open pollination the seed set on these plants ranged from a few seeds per head (about 5 per cent) to nearly 50 per cent. The remaining three plants grown which appeared to be pure *H. argophyllus* had pollen fertilities of 76 per cent or better and seed sets of over 90 per cent.

(3101). In 1948 plants of *H. argophyllus* and the red-rayed form of *H. annuus* were grown in a home garden. One plant which volunteered the next year was intermediate between the two presumed parents in pubescence but had the deep maroon rays approaching those of the *H. annuus* parent. The pollen fertility of this plant was 30 per cent and the seed set upon open pollination was about 10 per cent.

(4963). Reciprocal crosses made between *H. annuus* (seed from St. Louis, Mo.) and *H. argophyllus* (Bodger Seed Co.) produced 100 per cent seed set. The F_1 seedlings from the seed of the *H. annuus* parent died from some unknown cause soon after transplanting into the field, but five of the plants secured from the *H. argophyllus* parent reached maturity and were extremely vigorous. These F_1 plants bloomed in late July and early

August, intermediate between the blooming dates of the parental strains. The mean pollen fertility of these five plants was 33 per cent with a range of 30 to 36 per cent. Seed set upon open pollination varied from about 10 to 40 per cent.

Backcrosses. Backcrosses of 3101 were made to *H. argophyllus.* The pollen fertilities of the backcrosses were considerably higher than that of the F_1, ranging from 49 per cent to 64 per cent with a mean of 58 per cent. In appearance these plants were almost indistinguishable from the recurrent parent in pubescence, but 4 of the 6 plants showed some red in the rays which was derived from genes of *H. annuus.*

Backcrosses of the artificial hybrid (4963) were made to both parents. The backcrosses to *H. argophyllus,* 29 in number, showed some variation in the degree of pubescence approaching the F_1 on one hand and the recurrent parent on the other (fig. 2). The majority of the plants, however, could not be distinguished from the recurrent parent unless the plants were compared side by side. The pollen fertilities on these plants averaged 47 per cent with a range of 25 to 76 per cent. Seed set was studied in two plants and varied from 10 per cent to approximately 50 per cent. These backcrosses all came into bloom earlier than *H. argophyllus* but were two weeks or more later in flowering than the backcrosses to *H. annuus.*

Six plants of the backcross to *H. annuus* were grown. The pubescence of these plants was always greater than that of the recurrent parent (fig. 2), but not outside of the range of pubescence found in *H. annuus* as a whole. For example, plants of *H. annuus* which are fully as pubescent as these backcrosses have been grown from seed from both Missouri and North Dakota, and it seems unlikely that the pubescence of the plants in these regions could have been derived from hybridization. The pollen fertilities of these backcross plants were slightly less than that of the parent species, ranging from 15 to 99 per cent with a mean of 63 per cent. Seed set on two plants was only slightly less than that normally found in the "pure" species. These plants all came into bloom two to three weeks later than their *H. annuus* parent.

CYTOLOGICAL OBSERVATIONS

Both *H. annuus* and *H. argophyllus* have the haploid chromosome number of 17 (Heiser, 1949). Meiosis was studied in five F_1 plants (table 2). In the majority of the cells there were 13 to 15 bivalents and one or two chains, rarely rings, of four chromosomes (fig. 3). In addition to the configurations given in table 2, univalents were observed in six cells, and in a few other cells chains of six or eight chromosomes were seen. No evidence of bridges and acentric fragments was observed at anaphase. From these results it would seem probable that the two species differ by two translocations. The number of pairs formed in this hybrid is much higher than in any of the annual hybrids of Helianthus previously studied (Heiser, 1947, 1949, 1950).

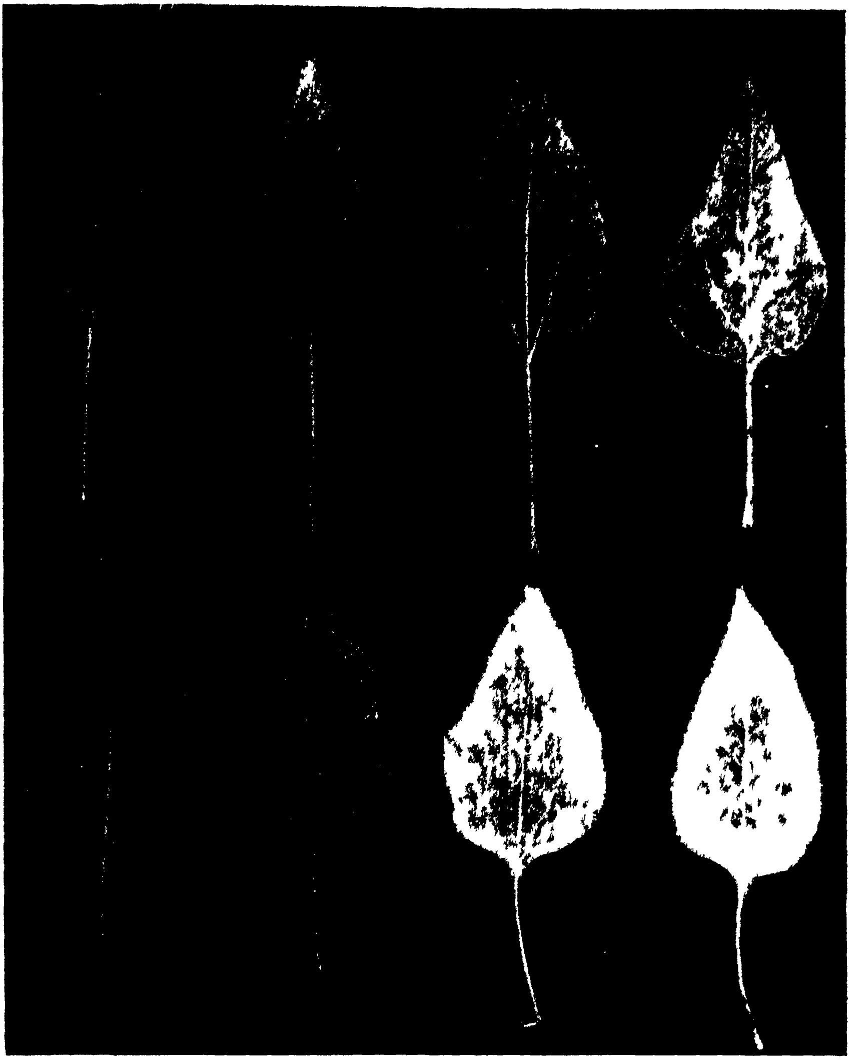

FIGURE 2. Leaves from greenhouse plants of *Helianthus annuus*, *H argophyllus*, and backcross plants. First column, *H. annuus;* second column, *H.* (*annuus* × *argophyllus*) × *annuus*; third column, *H.* (*annuus* × *argophyllus*) × *argophyllus;* fourth column, *H. argophyllus.* (reduced)

DISCUSSION

Helianthus annuus and *H. argophyllus* are obviously species of close relationship as is seen by both the morphological and cytogenetic analyses. As yet little is known of the origin of these two species, although it seems likely that they came from a recent common progenitor and have differentiated in geographical isolation. In all probability their distribution was originally

TABLE 2

PAIRING IN F_1 HYBRIDS OF *H. annuus* × *argophyllus*

Configurations observed	Number of cells in hybrid					Total
	#4817-1	#4817-2	#4963-1	#4963-5	#3101	
17 II's	3	4	3	3	1	14
15 II's, 1 IV	15	6	3	8	16	48
13 II's, 2 IV's	2	9	1	2	9	23

allopatric. Through man's agency *H. annuus* was introduced into eastern Texas, and the two species have since hybridized.

Asa Gray (1884) stated that *H. argophyllus* "degenerates in cultivation apparently into *H. annuus.*" Watson (1929), although realizing that *H. argophyllus* is a near relative of *H. annuus,* stated that there was no foundation for Gray's statement "that it hybridizes freely with *H. annuus,* or that it fades into the latter species along the northern limits of its range."

In all probability both Gray and Watson are correct to some extent, although Watson is attributing more to Gray than he actually wrote. In cultivation crossing may occur between these two species, and due to the fact that *H. annuus* is at an advantage in most garden soils it would seem likely that backcrosses to *H. annuus* would be the most likely to survive. Hence, *H. argophyllus* would "degenerate" into *H. annuus* through cultivation.

On the basis of limited field study and the study of herbarium material, no clearcut evidence of introgression in nature has been found, although it does seem possible that the occasional serrate leaf in *H. argophyllus* might have originally been derived from *H. annuus.* The hybridization experiments have shown that backcross plants approach very closely the recurrent parent in appearance. Moreover, in nature those backcrosses and other hybrid derivatives approaching the parental types would probably be most likely

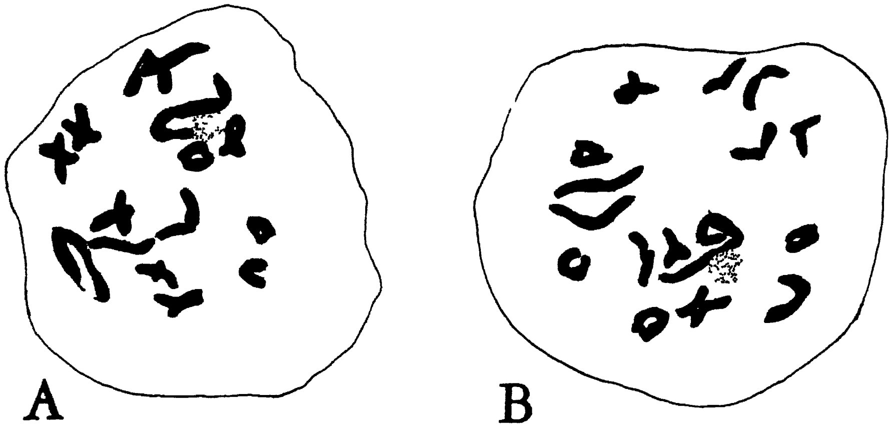

FIGURE 3. Camera lucida drawings of meiotic configurations in F_1 hybrid, *Helianthus annuus* × *argophyllus.* A, Cell showing 13 bivalents and 2 chains of 4 chromosomes each; B, Cell showing 15 bivalents and 1 chain of 4 chromosomes. X, 1280.

to survive. Hence, it would be exceedingly difficult to recognize the backcross plants in nature let alone to recognize introgressants. As Anderson (1949, p. 2) has written, "In all of the cases with which I am personally familiar, many, if not most, first and second backcrosses, if found in nature, would by taxonomists be accepted as varieties or slightly aberrant individuals of the species to which they were backcrosses." This statement certainly holds true for the backcrosses of *H. annuus* and *H. argophyllus*. It seems rather anomalous that these species show a higher degree of fertility than other annual hybrid combinations and at the same time the evidence for introgression is far less clearcut.

A third annual sunflower, *H. debilis* var. *cucumerifolius*, also occurs in eastern Texas, and like *H. argophyllus* is adapted to sandy soils. This species is known to hybridize with *H. annuus* (Heiser, 1950). No hybrids have been reported between *H. argophyllus* and *H. debilis* var. *cucumerifolius* although as yet critical field work has not been undertaken in southeastern Texas where such hybridization might be expected. The artificial cross between these two species has been made. This hybrid is very vigorous but rather highly sterile, producing from 1 to 21 per cent good pollen. In view of the fact that *H. argophyllus* and *H. debilis* var. *cucumerifolius* show the same general type of soil preference and occur in the same region, there must be other types of barriers preventing free hybridization between them. The difference in time of blooming between these species is rather pronounced, although there is some overlap, and probably is a rather potent barrier to extensive hybridization. The high degree of sterility of the F_1 hybrid, of course, would limit the amount of backcrossing. There may be other ecological barriers in addition, as yet unknown, which separate the two species.

If, as has been assumed, *H. annuus* is a recent immigrant in eastern Texas, its ability to spread and maintain itself in this region to which it was not adapted may be due to the acquisition of genes from *H. debilis* var. *cucumerifolius*, as previously suggested (Heiser, 1950), or from *H. argophyllus*. Watson is correct in stating that there is no evidence that the two species fade into one another.

No swamping is occurring as the result of the hybridization which is taking place because of the barriers imposed by the adaptation to different soil types, the partial sterility of the hybrids, and the partial difference in time of blooming. The ability of these entities to remain distinct in nature in spite of hybridization rightfully entitles them to the designation of species.

SUMMARY

Helianthus annuus and *H. argophyllus* are closely related species and natural hybrids between the two have been found in Texas. The artificial hybrid has been analyzed, and although showing some sterility, is more fertile than previously studied hybrids between other annual sunflowers. The artificial backcross plants approach very closely the recurrent parent in appearance, although they show some reduction in fertility. Analysis of

meiosis in the F_1 hybrid revealed 13 to 15 bivalents and one or two chains of four chromosomes in the majority of the cells. Whether introgression has occurred between these two species is not yet clear. Barriers imposed by the habitat, sterility and time of blooming have been effective in preventing the amalgamation of these two species under natural conditions.

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THE NEMATOCYSTS IN THE CTENOPHORE EUCHLORA RUBRA

Euchlora rubra (Kölliker) is known as the only ctenophore provided with nematocysts. As early as in 1856, Gegenbaur noticed the presence of nematocysts in this ctenophore. Chun (1880) also records and illustrates nematocysts in the same species in his magnificent monograph. Since then few workers, even authors of comprehensive works on Ctenophora, have mentioned this peculiarity in Euchlora. This is undoubtedly due to the extreme rarity of this ctenophore. In 1942 I had the chance of examining four specimens of this ctenophore collected at Seto in Wakayama Prefecture by Dr. T. Tokioka. On examining the sections of these specimens, I recognized numerous nematocysts in all of the specimens. The nematocysts were imbedded in the tissue of the tentacle along its lateral sides throughout the length. They were also found in the wall of the tentacular canal, on the side underlying the base of the tentacle. These nematocysts were of two kinds which might be called macro- and micronematocysts respectively. These occurred in mixture, macro- and micronematocysts roughly in proportion of 1:20–30. Thus, the occurrence in this ctenophore of the nematocysts is beyond question.

I reported this finding in two papers published in the same year. I had noticed, however, that the nematocysts were imbedded in the tissue of the tentacle, instead of being arranged on the surface of the latter. Especially, their occurrence in the wall of the tentacular canal appeared rather incomprehensible. More recently, at the suggestion of Dr. Libbie Hyman, I have become convinced that the nematocysts are of foreign origin, probably derived from some small medusae eaten by the ctenophore. This conjecture seems to clear up all the enigmas presented above.

It should be pointed out, however, that this ctenophore has no colloblast, and this feature is quite unique among tentaculate ctenophores. Possibly, this ctenophore uses its tentacles simply as balancing or floating apparatus, and captures its prey only with its large mouth. In any case, here is another case where nematocysts of foreign origin simulate their occurrence in an animal outside of the Cnidaria.

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PHYLLOTAXIS-LIKE ARRANGEMENT OF ORGANS AND ZOOIDS IN SOME MEDUSAE

Phyllotaxis is an order universally found in the arrangement of organs in higher plants. As far as the writer knows, no such order has ever been recognized in animals. For some years he has noticed the same order in the arrangement of organs in some medusae. For instance, the tentacles in the trachymedusa (or limnomedusa according to some authors) *Gonionemus depressum*, akin to *G. murbachii*, are of various length, and their arrangement appears quite irregular at first sight. More careful examination of specimens of various developmental stages, however, reveals a distinct order among these tentacles. The relative size of the tentacles in each quadrant shows the order of development. If this order is numerically designated, the tentacles of different sizes are arranged in each quadrant as: 1, 14, 6, 19, 11, 3, 16, 8, 13, 5, 18, 10, 2, 15, 7, 20, 12, 4, 17, 9, 1, where 1 stands for a perradial tentacle. No. 2, which should otherwise be the interradial tentacle, does not take the exact interradial position, but is shifted clockwise to some extent in the subumbrellar view. Likewise, no. 3 and no. 4, which should be the adradial tentacles, are shifted somewhat, the former anti-clockwise and the latter clockwise. The same displacement may be found in other tentacles also. All these seeming irregularities are due to the phyllotaxis-like arrangement, instead of the usual radially symmetrical arrangement, of these tentacles.

In phyllotaxis, the divergence between consecutive organs (leaves, branches, flowers, etc.), in most cases, coincides with one of the following fractional series: $\frac{1}{2}$, $\frac{1}{3}$, $\frac{2}{5}$, $\frac{3}{8}$, $\frac{5}{13}$, $\frac{8}{21}$, $\frac{13}{21}$.... This series has its limiting value in 0.382 which corresponds with 137° 30′ 28″., if the whole circumference of the stem is taken as 360°. In this ideal case, the organs should take the arrangement just mentioned. In the case under consideration, however, the quadrant of the umbrella makes a unit, and the divergence between consecutive tentacles is $\frac{1}{4}$ of 137° 30′ 28″, namely, 34° 45′ 14″.. Yet, the arrangement of the tentacles follows the same order. A new tentacle, according to the scheme, should divide the arc between the preceding tentacles into the ratio of 1 : 0.618. Actual measurements have revealed this to be 1 : 0.58–0.61.

The same order may be found in the arrangement of the ex-umbrellar tentacles of another trachymedusa, *Olindias formosa.* These tentacles are situated on various levels quite without order at first sight. More careful examination reveals that they are also in accordance with the order in phyllotaxis. This medusa also has numerous centripetal canals on the subumbrellar side, which start from the circular canal and terminate on various levels at some distance from the manubrium. The arrangement of these canals accords with the same order. The same scheme may also be found in the arrangement of tentacles of the anthomedusa *Misakia typica,* which is allied to Proboscidactyla and Willia.

Lastly, the dactylozooids in the siphonophore *Porpita umbella* are arranged much like the tubuliform flowers of the Compositae on the head disc. Undoubtedly, the mode of arrangement of these zooids and flowers are subject to the same order.

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ERRATA

Komai, Taku: In Sept.-Oct., 1950, issue first line, p. 391, should read: "schmidt, 1944, 'Science in the University,' pp. 183–210, gave several instances of crowd-"; on p. 392 the reference of Stem, C., should read, "1930. 'Multiple Allelie,' Handb. Vererb. 1."

Gregg, John R.: In Nov.-Dec., 1950, issue, p. 419, line 4, insert "own" after "his"; p. 421, line 15, delete last word in line, "of"; p. 422, line 25, change "contrary" to "subcontrary"; p. 435, line 3, insert "and" after "obtained."

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INHERITANCE OF CORN GRASS A MACROMUTATION IN MAIZE, AND ITS POSSIBLE SIGNIFICANCE AS AN ANCESTRAL TYPE*

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INTRODUCTION

From time to time mutations arise which have a more pronounced effect upon the organism than the ones usually studied in genetic investigations. Goldschmidt (1940, 1948) refers to these as macromutations in contrast to the more abundant micromutations. He was the first to emphasize the importance of macromutation in evolution. He questions whether the normal mutations with which we are all familiar, the micromutations, have much if anything to do with the evolutionary process.

Corn grass is definitely such a macromutation. A single gene changes the morphology of the maize plant so completely that it is scarcely recognizable as corn. The leaves are narrow, the plant tillers profusely, the pistillate flowers are located in the axils of the leaves with but a few flowers in a place, and not in a well-defined ear (figures 1, 2 and 3). When first grown the Corn grass plants either had no staminate flowers at all or the flowers were so enclosed in long bracts that no pollen was ever shed. It was maintained by saving open pollinated seed from the heterozygous Corn grass plants. Since the gene is dominant such plants usually segregate in a 1: 1 ratio.

Corn grass plants can be propagated vegetatively. A single clump has been divided into sixteen plants and undoubtedly more could have been obtained. This character, along with the seed distribution among many small "ears" on the plant, are two factors that might permit such a plant to become established in the wild state. In a climate free from frost it might be a perennial. Such a plant could then go on for generations with a likelihood that eventually a reverse macromutation might occur and a typical corn plant be produced. To some this may seem too easy a solution to the question of how corn originated. We are not postulating this as the answer, but such a possibility is certainly not unreasonable. In thinking

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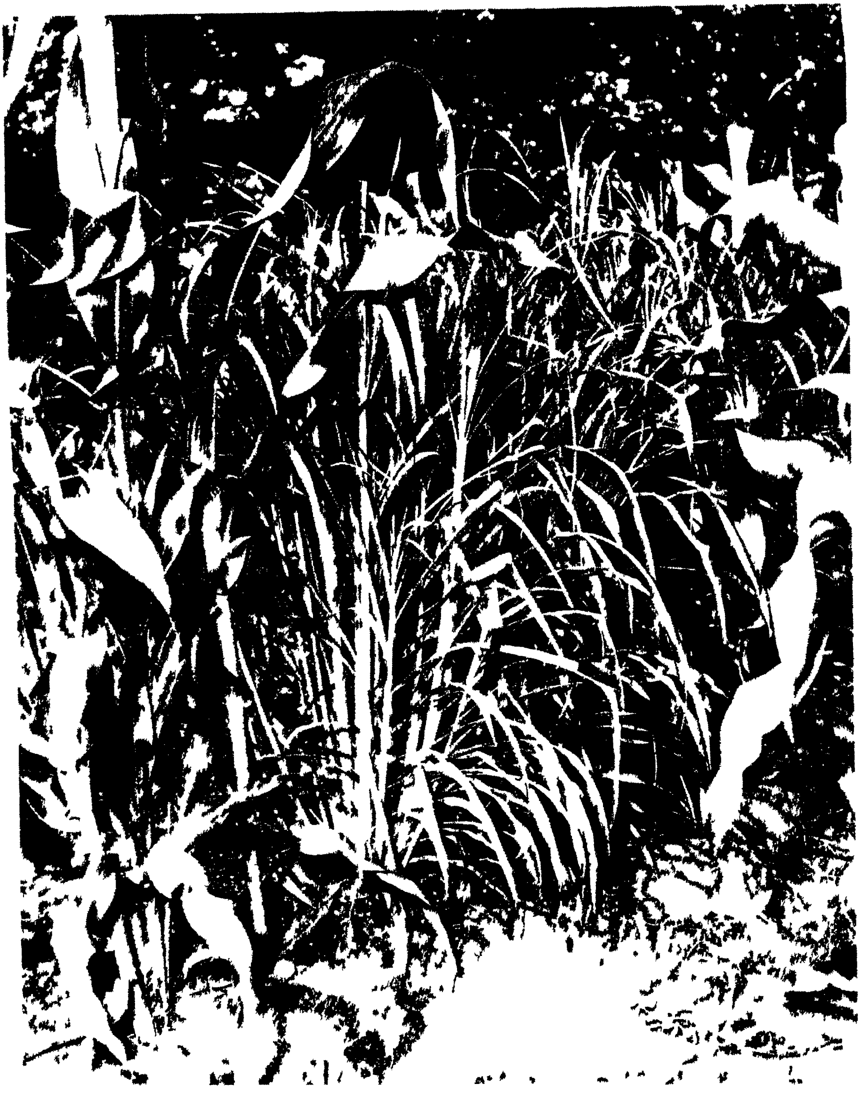

FIGURE 1. Corn grass and normal plants in a segregating progeny. Corn grass plants produced a few seeds each, but no pollen. Summer of 1948.

about the ancestor of corn perhaps we have been thinking too much along the line of a corn plant only slightly modified by a series of micromutations, when we should be considering an entirely different type of plant from which corn arose by a single macromutation. If a single gene difference can make plants as dissimilar as corn and Corn grass there is no reason to believe that in some prehistoric time a profusely tillered, perennial, narrow-leaved grass-like plant might not have mutated to a single stalked corn with a well-defined ear similar to types we have today.

HISTORY OF CORN GRASS

A single plant of Corn grass was first discovered in the summer of 1941 in a field of Lincoln sweet corn hybrid on the farm of A. W. Winter, Passaic County, New Jersey, by Bailey Pepper, entomologist at the New Jersey Agricultural Experiment Station. Dr. Pepper was dusting the experimental plots of sweet corn for control of the European corn borer. He was so struck with the unusual character of the plant that he transplanted it to the greenhouse at New Brunswick and matured a 'small quantity of seed, which was given to Dr. C. M. Haensler, pathologist at the New Jersey Station. Dr. Haensler in turn gave it to me.

It was grown for the first time on the farm of the Connecticut Agricultural Experiment Station in 1945 and was segregating for both *Cg* and normal plants in about equal numbers. The normal plants upon selfing produced all normal offspring, while open pollinated seed of the Corn grass gave normal and *Cg* again in about equal numbers. The Corn grass plants were heterozygous and had received pollen from recessive normal plants.

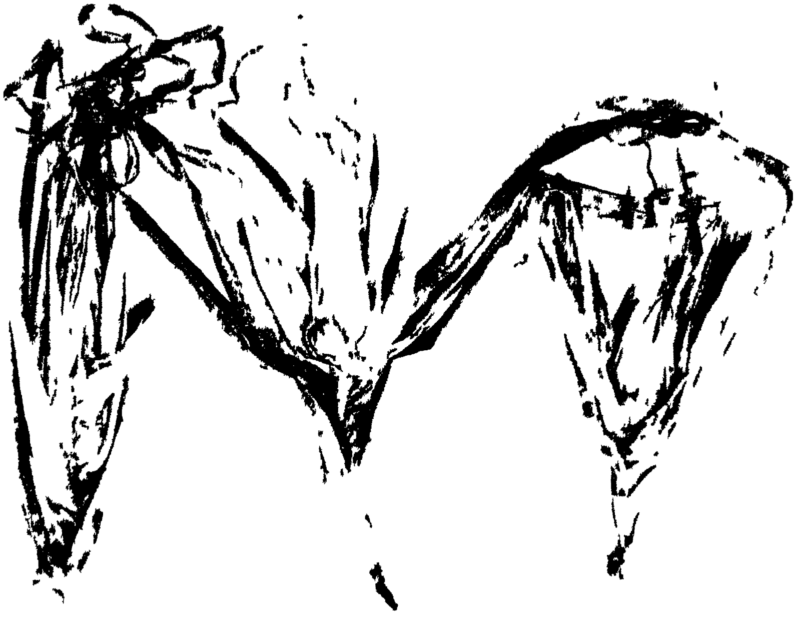

FIGURE 2. Three fairly typical "ears" of Corn grass. Such ears may contain from 0–20 seeds with an average of seven seeds each. These ears were produced on plants similar to those in figure 1. Summer of 1948.

When first reported Corn grass was referred to as a "second Teopod" (Singleton, 1947) because of some similarity to the original Teopod (Lindstrom, 1925). However, Corn grass is much more striking in appearance than the original Teopod, which would never be mistaken for anything other

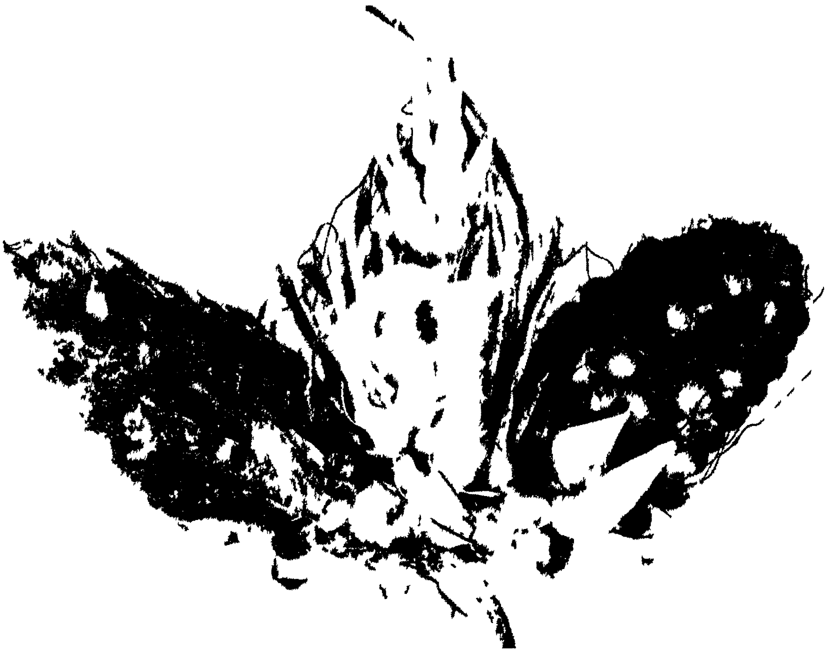

FIGURE 3. Three ears produced on a single plant, greenhouse crop of 1948 autumn sowing. Note extreme variation in development of bracts in three ears produced on the same plant.

than corn. The leaves of Teopod are broad and the stalk is about the same height as normal corn. Both inflorescences are considerably modified, however. The seeds are enclosed in long bracts, with the ear much shortened and reduced in size. The tassel of Teopod has few branches and often has a small ear at the base of a single staminate spike. Corn grass, on the other hand, has very narrow leaves unlike normal corn and has much smaller ears with no tassel on many of the plants. Consequently the name of Corn grass seems more appropriate than a "second Teopod." Also the term "second Teopod" should not have been used by us (1947) since Lindstrom (1935) reported two additional cases of mutations to a Teopod type plant. This publication of Lindstrom has but recently come to our attention. One of the illustrations of the new Teopod of Lindstrom appears quite similar to Corn grass. Lindstrom stated "Crosses of the new Teopod-like variants with the original stock of Teopod, indicate that the same basic dominant *Tp* gene is involved." He did not elaborate further on this statement or present evidence of segregating progenies of these crosses, so it cannot be concluded from the data presented that all three of Lindstrom's Teopods were due to the same gene. One was much more similar to Corn grass than to Teopod. Crosses of Corn grass and the original Teopod of Lindstrom have demonstrated they are not due to the same gene. Evidence is presented later in this paper.

EXPERIMENTAL RESULTS

When Corn grass was first grown at the Connecticut Experiment Station in 1945 it was noted that there were about equal numbers of Corn grass and normal plants in the progeny. Since it was not known whether the mutant was dominant or recessive, seed was saved from both types; selfed seed from the normal plants, and open pollinated seed from the Corn grass plants which produced no pollen. Such open pollinated seed represented a backcross progeny *Cg*/+ × + and produced a progeny segregating for approximately 50 per cent. Corn grass plants. Selfed seed from the normal plants produced only normals, the recessive type.

It was noted in several years that other segregations gave approximately 1 : 1 ratios. Typical of these are the data for 1950. In these progenies a search was made for a linkage of Corn grass with the following genes, *su, pr, r, wx* and *gl*. No linkage was found with these genes in five different chromosomes. It is significant that none was found with *gl*, which shows only 9.5 per cent. crossing over with Teopod, another bit of evidence indicating Teopod and Corn grass are determined by different genes.

TABLE 1

RATIOS OF *Cg* AND + PLANTS IN BACKCROSSED PROGENIES (*Cg*/+ × +) 1950

Type of Seed	+ Plants	*Cg* plants	Total
Pr Su	58	60	118
Y Su wx	39	27	66
pr Su	46	60	106
su	48	45	93
Y Su Wx	52	56	108
	243	248	491
Expected	245.5	245.5	

A chi square test ($\chi^2 = .05$) is unnecessary to tell us there is no serious deviation from a 1 : 1 ratio.

Table 2 shows F_2 segregations in which *gl* and *Cg* were involved.

TABLE 2

SELFED PROGENIES SEGREGATING *Gl Cg*/*gl cg*

Row No.	*Cg Gl*	*Cg gl*	*cg Gl*	*cg gl*	
931–950	174	48	55	15	
955–964	59	21	25	6	
878–889	42	17	22	6	
Total	275	86	102	27	490
Expected	275.6	91.9	91.9	30.6	

Here again there is no significant variation from a 9:3:3:1 ratio indicating that *Cg* is not linked with the gl_1 locus, evidence that *Cg* and *Tp* are not allelic. Conclusive proof of this however was obtained from the cross of *Tp*/+ × *Cg*/+. *The* F_2 of this cross gave the following segregation:

TABLE 3

SEGREGATION OF F_2OF *Tp*/+ *Cg*/+

Row No.	*Tp Cg*	*Tp*+	*Cg*+	++	
901–905	18	11	12	10	
908–915	19	53	64	29	
Total	37	64	76	39	216
Expected	121.5	40.5	40.5	13.5	

In this instance there is a wide deviation from a 9:3:3:1 ratio. Possibly there is a linkage between *Tp* and *Cg*. If so *Cg* must lie to the right of *Tp* since *Cg* and *gl* show no linkage. Part of the apparent linkage may be due to faulty classification. It was extremely difficult to distinguish *Tp*+ and *Tp Cg*. Perhaps we were overcautious in not putting as many in the double dominant class as it deserved. The basis of classification of *Tp Cg* plants was a modification of the *Tp* type of plant. Plants assumed to be *Cg Tp* had a longer ear than ever found on any *Tp* plant without *Cg*. (figures 4, 5 and 6). There was less difficulty in classifying the normal (++) plants although it is possible for modifying genes to affect a *Cg* plant to such an extent that it appears normal. This will be discussed more fully in another section. If the excess of + plants is a real one and not due to modifiers it can scarcely be attributed to linkage since the genes entered the cross in the repulsion phase. The fact that any *Cg Tp* and ++ plants appeared in the F_2 progeny is direct evidence that *Cg* and *Tp* are not allelic. More experimental data are needed to establish a linkage of *Cg*. No indication of linkage was found in tests with genes on the following chromosomes, 4, 5, 7, 9 and 10. The other five chromosomes have not been tested.

ENVIRONMENTAL EFFECT ON MORPHOLOGY OF CORN GRASS

Corn grass grown in the greenhouse in the autumn with no supplemental light is considerably modified in its morphology, assuming an appearance more like normal corn (Singleton, 1949, 1950). Plants from such fall sowings assume a more upright growth, have fewer tillers, produce tassels (in some instances almost like normal corn) and have much broader leaves than field-grown plants. Some of these *Cg*/+ plants grown in the greenhouse have been mistaken for normal corn. Upon selfing, these plants have segregated for *Cg* and + thus demonstrating they were genetically *Cg*, also

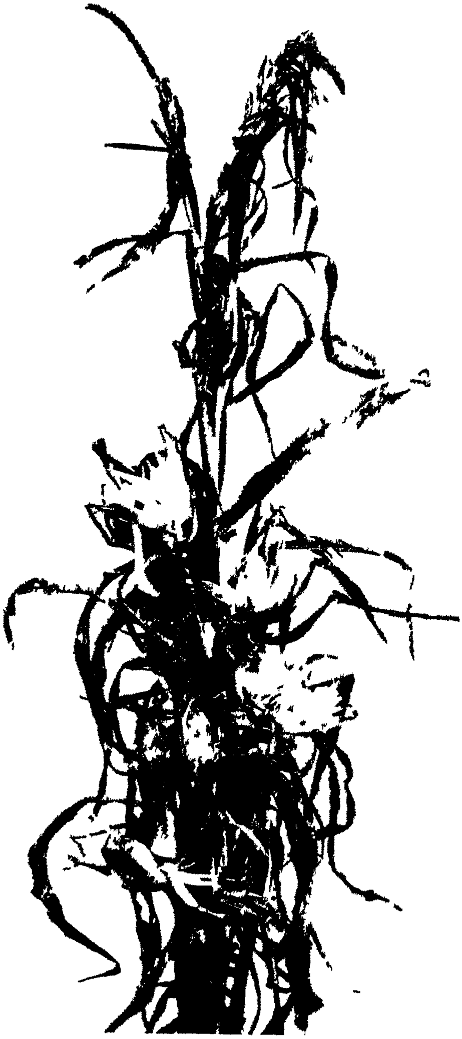

FIGURE 4. Plant heterozygous for both Teopod and Corn grass.

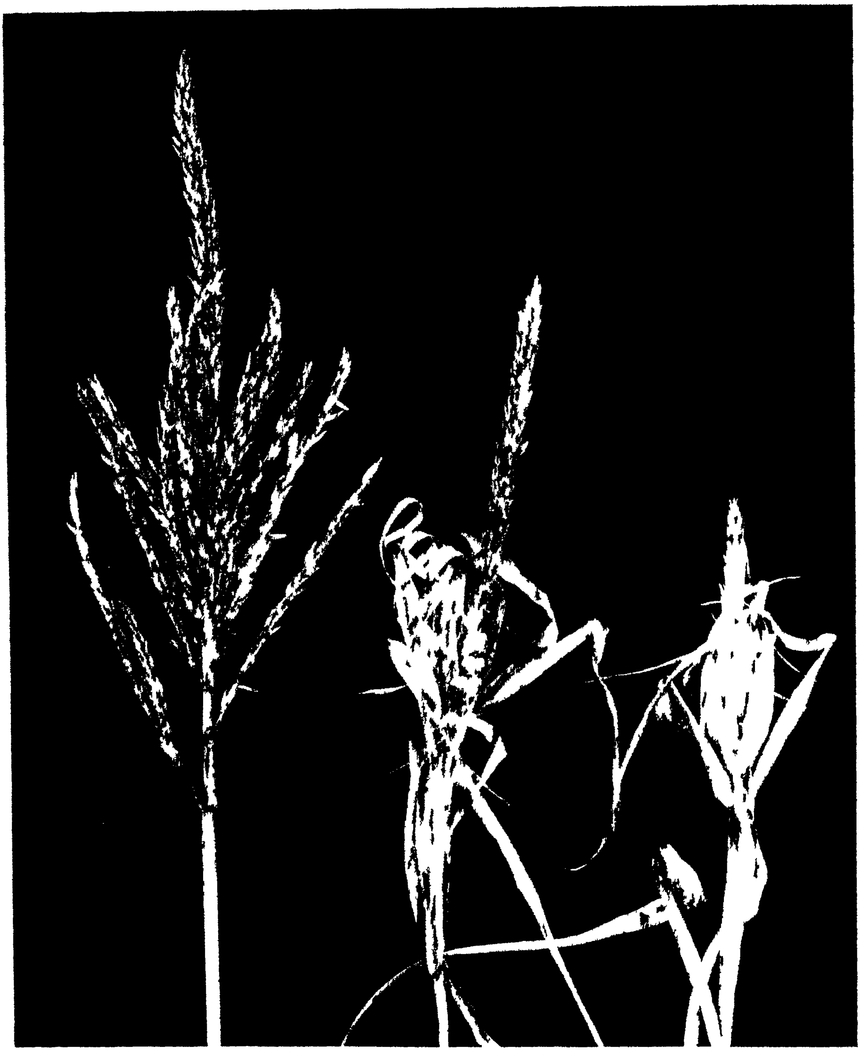

FIGURE 5. Three tassels on *Tp+Cg+* plant illustrated in figure 4. Note considerable variation in tassel development.

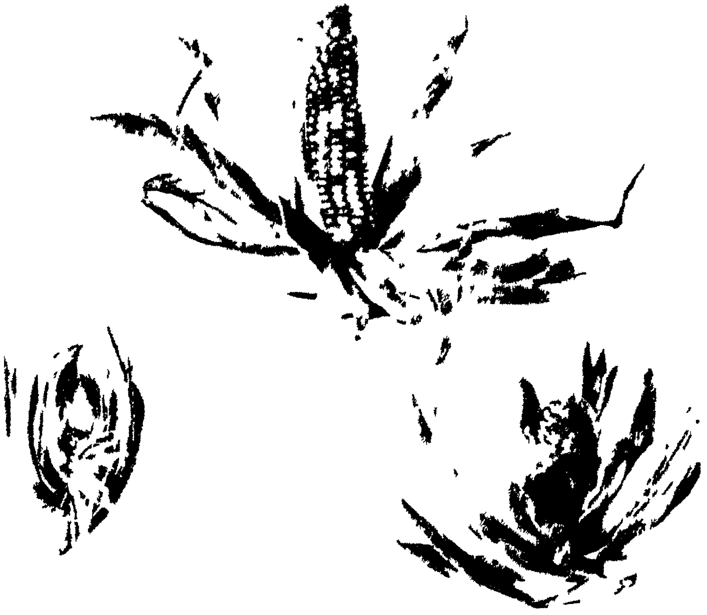

FIGURE 6. Three ears on a *Tp+Cg+* plant shown in figure 5. Note variation in ears, also one ear considerably longer than those produced on *Tp/+* plants without influence of *Cg* gene.

showing how completely the Corn grass plants can be modified, presumably by external growing conditions. It seems that light may have been largely responsible for this marked change, since the plants were grown in the autumn when the days were becoming shorter. The return to a Corn grass-like plant in the January sowings further suggests light, rather than temperature as the controlling factor. The temperature of the greenhouse was thermostatically controlled and remained fairly constant throughout the growing season. However, in Texas, greenhouse-grown plants were upright with few tillers even though supplied with supplemental light (Whaley and Leach, 1950). Their statement seems pertinent, "The relative roles of light and temperature in this tillering response remain to be worked out."

This unusual type of Corn grass plant was first observed in the fall of 1947 when a few plants were grown in the greenhouse of the Connecticut Experiment Station at New Haven. The following year sowings were made at intervals of two weeks beginning September 15 at the Brookhaven Laboratory. This experiment was repeated in 1949 with similar results. Plants from the September sowings were similar to the summer type plants. In October the more erect upright growth commences and persists until about January 1 when the summer type growth reappears. There is a somewhat gradual transition from the erect type to the typical Corn grass plants. This is illustrated in figures 7, 8 and 9. Figure 10 shows a more striking change that took place in 1949. In this instance the erect plants were more nearly like normal corn.

It is remarkable that such a small change in the environment could produce such a gross morphological effect. Not only is the size and number of tillers affected, but in certain plants a potential floral structure is changed into a completely vegetative part (figures 11 and 12). This is quite characteristic of summer type Corn grass. The change illustrated here occurred on the January 1, 1949, sowing.

GENETIC MODIFIERS OF CORN GRASS

Not only environmental but also genetic modifiers have a pronounced effect on the Corn grass phenotype. When first grown in 1945 and for several years thereafter Corn grass in the summer time never shed any pollen. Any anthers if present were completely enclosed by long bracts and hence produced no pollen. In 1949 and 1950, however, we have had plants that produced fairly normal tassels, though small, and produced plenty of viable pollen. Apparently modifying genes have been introduced into the stock. Mr. Walton Galinat, who has made a comprehensive study of the morphology of Corn grass, has observed these modifiers also. Galinat (1951) crossed Corn grass by stocks differing markedly in maturity and has extracted widely

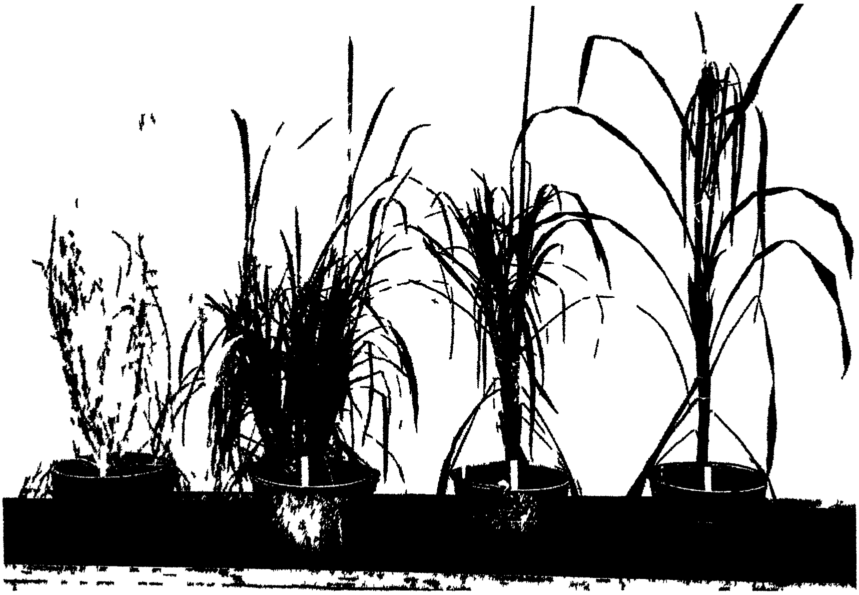

FIGURE 7. Corn grass plants from four different sowings (from left to right) September 15, September 30, October 15 and November 1, 1948. Note more upright growth in later sowings.

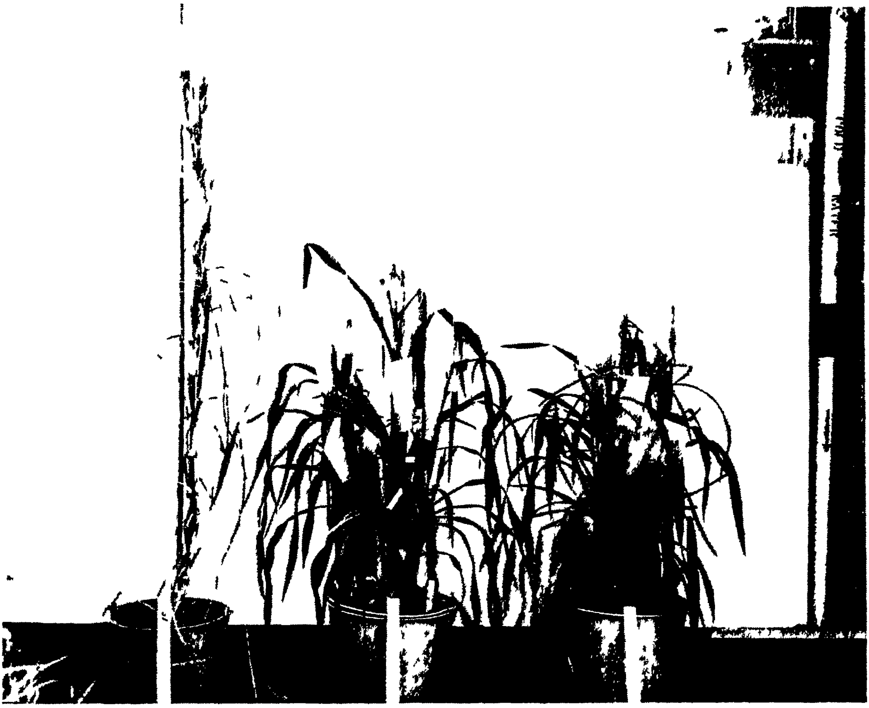

FIGURE 8. Corn grass plants from three sowings, November 15, December 1 and December 15, 1948.

divergent types of Corn grass with striking differences in ear and tassel development when grown under field conditions. Some of his Corn grass plants have produced ears and tassels comparable to normal corn. Apparently the Corn grass character is quite susceptible to modification and is fruitful material for a morphological study.

DISCUSSION

Corn grass is a mutant type whose morphology is grossly altered by a single gene. Meiosis is regular with no indication of chromosomal irregularity. Young plants of Corn grass more closely resemble certain species of common grasses than they do *Zea Mays L.* In fact, Corn grass is so different from corn, especially in the early stages, that students in taxonomy have failed to recognize it as a relative of corn. In Texas, it was so similar to Johnson grass (a serious weed) it was difficult to keep the farm hands from pulling out the Corn grass (Whaley and Leach, 1950).

The question arises whether such mutations as this and the somewhat similar Teopods have any bearing on the question of the type of plant that was the ancestor of maize. In our search for the ancestor have we been looking too diligently for a corn plant only slightly modified, when perhaps our attention should have been focussed on a grass-like plant such as the Corn grass with its grossly altered morphology? If a difference as large as this can be due to a single gene is there any reason to suppose that even greater differences might not be possible? If to Corn grass we add complete fertility in the homozygous condition along with a perennial habit of growth we have what might be considered an excellent ancestral type. And there are good reasons to believe that species may originate by single macromutations such as this rather than by a series of micromutations. Goldschmidt (1948) has presented the arguments for the importance of macromutations in evolution. We should like to quote Goldschmidt's closing paragraph (1948), "But it will be beneficial to realize that, in view of the failure of Neo-Darwinism to explain evolution occurring above the level of the ecospecies, science will be better served by attacking these problems with an open mind instead of from the standpoint of a school sworn to an inflexible dogma."

Both Weatherwax (1950) and Mangelsdorf (1950) have presented excellent dissertations on the history and the mystery of corn. Both have made extensive studies regarding the ancestry of corn and they do not agree entirely, which is natural when it is considered that no wild type of corn plants have been found. Mangelsdorf is inclined to think of the ancestor as a

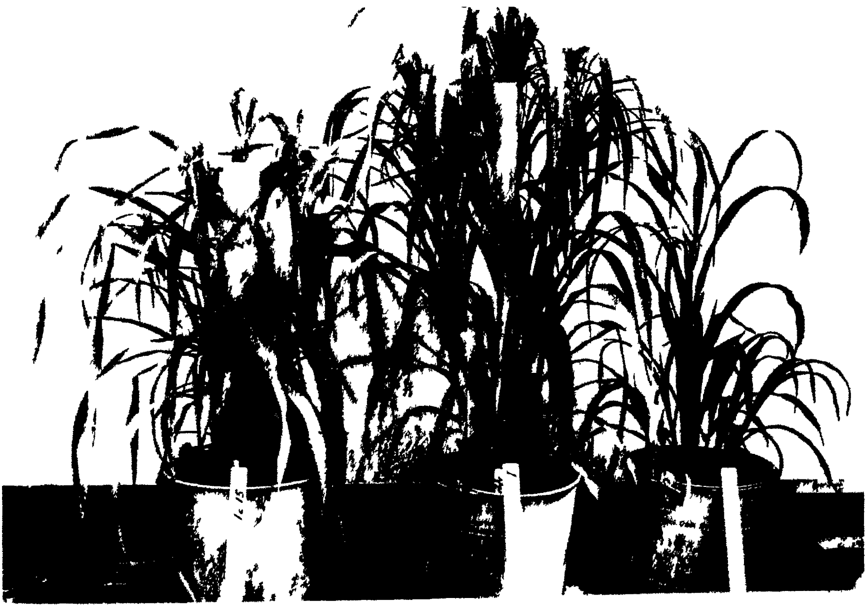

FIGURE 9. Corn grass plants from three sowings, December 15, 1948, January 1 and January 15, 1949. Plants from January sowing have all appearances of summer grown plants.

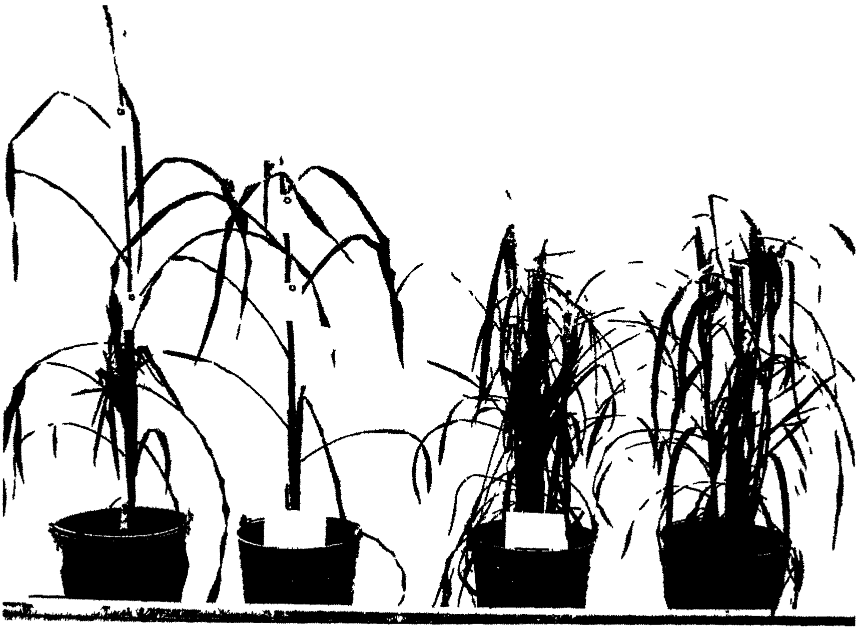

FIGURE 10. Two Corn grass plants on left from October 1, 1949 sowing, those on right from October 15 sowing.

tunicate popcorn being modified from normal corn by the single *Tu* gene. We would like to quote one paragraph (Mangelsdorf, 1950):

> So the teosinte theory has become increasingly untenable. Meanwhile the theory that corn originated from pod corn has become more and more plausible. When a modern hybrid form of pod corn is inbred (a process that usually intensifies inherent traits) the result is a plant quite different from ordinary cultivated corn. The ear disappears and the kernels, now borne on the branches of the tassel, are enclosed in glumes, or chaff, as in other cereals. This pure pod corn possesses a means of dispersal, since its seeds are not on a heavy ear but on fragile branches. In the proper environment it could undoubtedly survive in the wild and reproduce itself. It has characteristics like those of many wild grasses; indeed, in its principal botanical features it is quite similar to its wild relative Tripsacum. Pure pod corn has virtually all of the characteristics we would expect to find in the ancestral form of corn. Furthermore, it is more than a relative of corn; it *is* corn—a form of corn that differs from cultivated corn in exactly the way a wild species ought to differ from its cultivated counterpart. Finally, all the hereditary differences between pod corn and cultivated corn are traceable to just one gene on one chromosome. Thus a single mutation can change pod corn to the non-podded form, and it has actually done so in my cultures.

If we were to change the term "pod corn" to Corn grass in this paragraph we believe an even stronger case could be made for the origin of corn. Seed dispersal of Corn grass would be more easily accomplished since the "ears" are much smaller. Also Corn grass can be propagated asexually. The only thing we would have to change in Mangelsdorf's paragraph would be the last

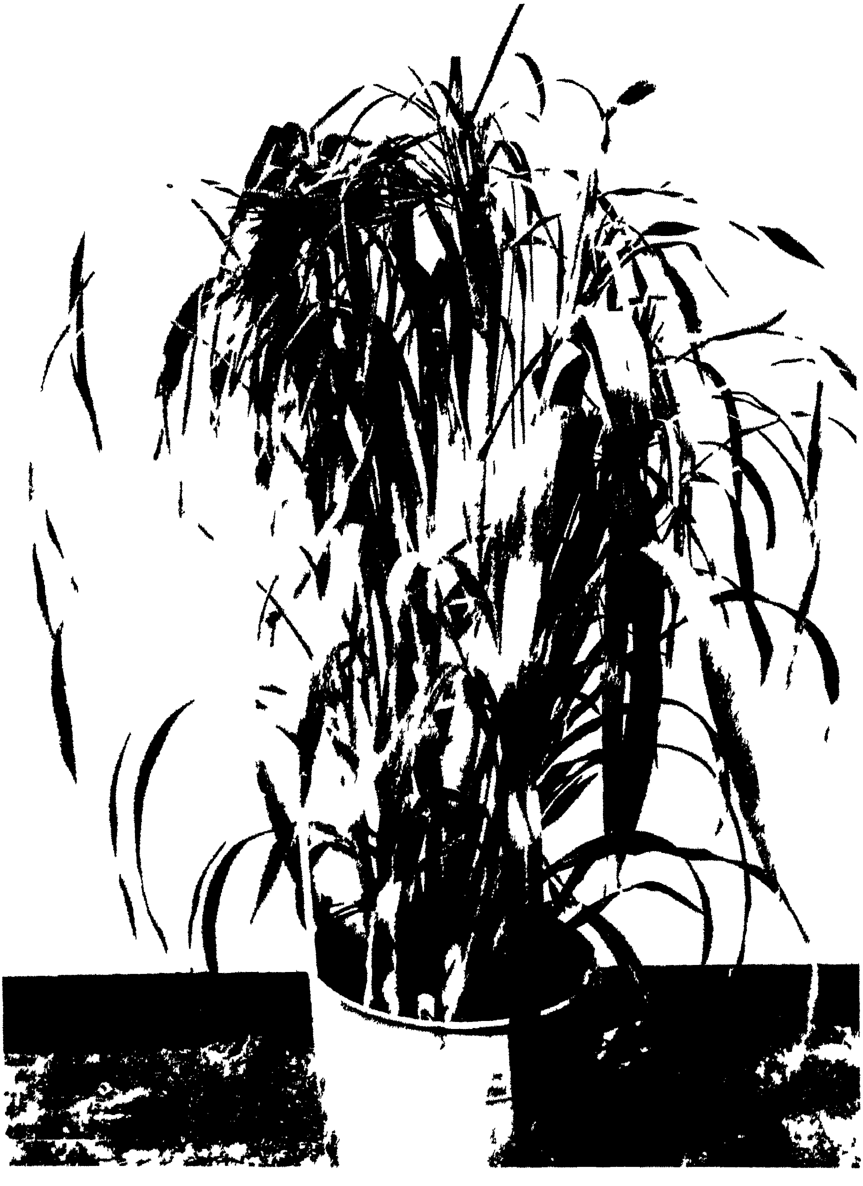

FIGURE 11. Plant of January 1, 1950 sowing showing summer type growth, also showing conversion of floral structure into a vegetative one. Larger view of this in figure 12.

sentence since we have not as yet secured a reverse mutation from Corn grass to normal. Such should be possible as soon as *Cg* is made homozygous. Preliminary attempts to induce somatic mutations from *Cg* to normal by use of beta radiation from P^{32} and gamma radiation from Co^{60} did not induce any mutations. Since somatic mutations occur less frequently than germinal ones it is not surprising that no mutations were found. If the homozygous *Cg Cg* is not sterile it should be possible to secure a reverse mutation to normal as the mutation must have occurred in the first place, if our theory is correct.

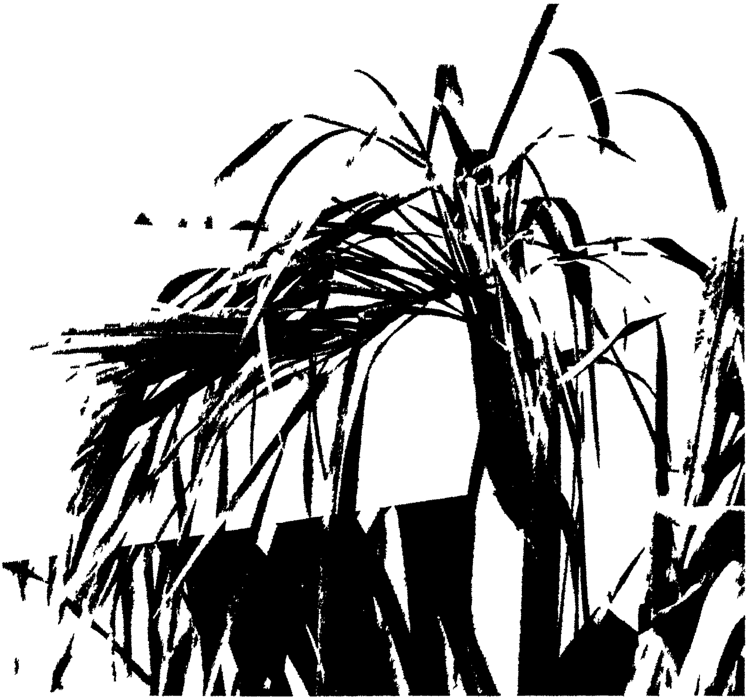

FIGURE 12. Tassel branch of plant of January 1, 1950 sowing showing transformation of a floral structure to a vegetative one. This type of transformation is quite common in plants grown in the summer in the field.

Another cogent reason for assuming that our corn as we know it arose by a single macromutation is that corn is absolutely dependent upon man for support. The primitive Indian would not have made any effort to select among grass-like plants those with a few more seeds than others. However, if a macromutation occurred it would have been recognized immediately and the chances are fairly good that it would have been cared for and propagated. Although it is not possible to obtain proof for this theory of the origin of corn (or any other theory for that matter) it seems possible that a Corn grass-like plant may have been the immediate ancestor.

SUMMARY

Corn grass is a monogenic dominant type of maize that arose by a single macromutation. It has narrow leaves, many tillers and under field conditions hardly ever sheds pollen. It can be propagated asexually and seed is distributed over the plant in many small ears containing but a few seeds each. Several of these characters are those which might be expected in an ancestor of maize. The possibility that a Corn grass type of plant was the ancestor of maize is discussed.

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ANATOMY OF GENE CENTERS[1]

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The geobotanical work of Nikolai Ivanovich Vavilov is familiar to nearly every biologist. The tremendous mass of material assembled by the institute which he directed for many years made possible a geographic survey of crop plants on a scale which has never been duplicated. In no other country and at no other time has an agronomist had such facilities at his disposal. The work is so monumental that it stands today neither substantiated nor disproved by independent research. Some of his claims have been questioned, but the principal theses of Vavilov are known and appreciated the world over, with the possible exception of his native land where his genius inspired jealousy and his integrity invited liquidation. During the greater part of 1948, the writer was privileged to travel through Turkey on behalf of the Division of Plant Exploration and Introduction of the U. S. Department of Agriculture. This is an area of considerable importance according to the studies of Vavilov (1926, 1935, 1940), and the purpose of this essay is to report certain observations made in the field and to call to the attention of American naturalists some of the opportunities now available for fundamental studies on the problem.

Vavilov's ideas have themselves undergone an evolution of considerable interest. In his classification of 1926 Asia Minor was included in the Asiatic, Mediterranean, Balkan, and Transcaucasian gene centers of wheat including the vulgare, durum, and monococcum groups, and of barley, rye, oats, peas, lentils, chickpeas, bittervetches, vetches, broadbeans and flax. In his classification of 1935 he established eight basic world centers in which most of the varietal wealth of our major crop plants are concentrated. He pointed out that these eight primary centers are separated one from the other by great deserts or mountain ranges. Only in Asia Minor do two basic centers overlap. As a result this part of the world is immensely rich in varietal resources of nearly a hundred species of cultivated plants. By 1940 in one of the last of his essays he had broken up these immense areas into a number of smaller centers of "agro-ecological groups." Of these smaller centers of diversity, Turkey is included at least to some degree in the Syrian group, Anatolian group, Armenian Xerophytic Mountain group, Caucasian Mesophytic High-mountain group, Transcaucasian Humid Subtropical group and the Mediterranean group. These later groupings are the result of many years of agronomic analyses of the vast amount of material previously accumulated, and of continued explorations of certain primary areas.

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[1] Contribution of the Division of Plant Exploration and Introduction, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture.

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It is to be noted that these agro-ecological groups do not coincide exactly with the original concept of center of origin. They represent a further breakdown, a further refinement in the geobotanical pattern of variation. Thus in the overall concept of Vavilov there appears to be a structure in the variation pattern which in itself is subject to study. Observations made by the writer in the field have led him to conclude that the variation pattern may be still further divided into discrete units of relatively small size some of which are intensely active from an evolutionary point of view.

A THRACIAN WHEAT FIELD

An examination of a wheat field in Turkish Thrace provides an education in crop evolution. The typical wheat field here contains a wonderful mixture of forms which according to some classifications would comprise a number of species, many botanical varieties, and dozens if not hundreds of agronomic varieties. Species of Triticum found in a single field might include *T. vulgare, T. durum, T. polonicum, T. compactum, T. monococcum, T. spelta, T. turgidum* and others (Gōkgōl, 1939). Several so called botanical varieties of each may be present and the agronomic variations are legion. Around the borders of the fields, in the weedrows, the roadsides, the waste spaces and to some extent in the fields themselves, the wild wheat relatives are found in abundance. The borders of many fields are actually carpeted with forms of Aegilops, Haynaldia and wild forms of Secale and Triticum. The remarkable array of variants closely resembles certain composite cross populations artificially generated by some of our agronomists in this country, but can hardly be explained on the basis of mechanical mixture alone.

Two population studies in barley may be cited to illustrate the nature of the Thracian wheat populations. In the first study by H. V. Harlan and M. L. Martini (1938) an artificial mixture of varieties was grown for successive generations at widely different localities in the United States. At most stations one or two varieties dominated the mixture within a very few generations, while at one or two stations especially suited to barley culture, all components of the mixture were present after more than ten generations. All varieties were recognizable throughout the experiment and *no new forms were observed.* In the second study (Harlan, Martini and Stevens, 1940) composite cross populations were obtained by crossing 29 varieties in all possible combinations. These populations carried in bulk for eight generations were exceptionally variable and so many new forms and variants were found that they were described as an "understory" in the population. Hundreds of altogether new combinations and some dozens of characters never before observed by agronomists were detected in the advance generations of the composite cross populations (Martini and Harlan, 1942).

GENE MICROCENTERS

With this experimental evidence in mind it seems readily possible to distinguish populations representing mechanical mixtures from those which are to some degree at least actively interbreeding. The Thracian wheat populations appear to be in the latter category. Most wheat populations throughout Turkey, however, belong to the first category being simple mechanical mixtures resulting from a primitive husbandry. Pure fields are rarely found in Asia Minor, but typical grain fields throughout most of Anatolia are represented by a mixture of several forms readily recognizable throughout a local region. The wheat populations of Turkish Thrace are of a different order and represent a remarkable accumulation of variant forms which one might refer to as a gene microcenter.

A similar microcenter was observed in the Kars basin near the Russian border containing entirely different sorts of wheats. The wild relatives consisted mostly of forms of rye and the wheats included *T. persicum* which according to Vavilov is characteristic of the Caucasian Mesophytic High-mountain group of wheats. *T. dicoccum* is more important in the high Kars basin as a secondary crop than *T. monococcum* which is grown on a large scale in Thrace. A third microcenter was observed in southeastern Anatolia in the upper Mesopotamian plain and extending into the Antitoros mountains. All three gene microcenters were characterized by the highly variable type of populations described for Thrace which contrasted sharply with the normal wheat populations throughout the remainder of Turkey.

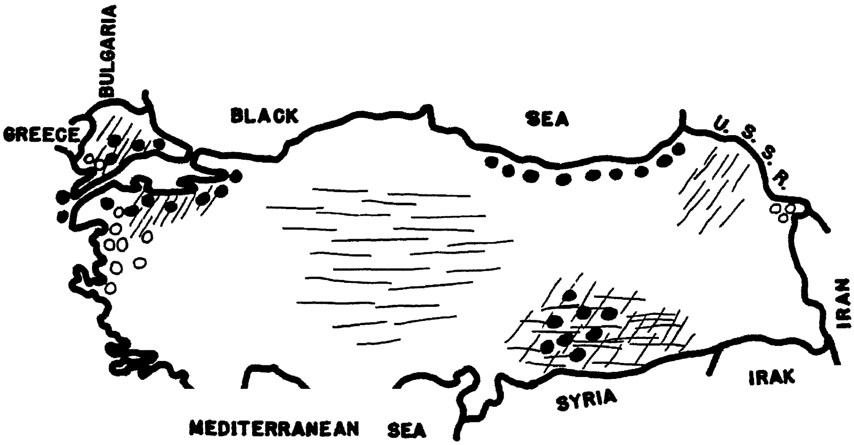

FIGURE 1. Some Turkish gene microcenters:

///// Wheats
ᴼcᶜo Muskmelons
—— —— Pumpkins, cushaws, watermelons, cucumbers, gourds
● ● ● New world beans

MICROCENTERS IN OTHER CROPS

Analogous variation patterns were evident in a number of other crop plants, table 1. The locations of microcenters for some of these crops are indicated in figures 1, 2 and 3. It is of special interest to note that several New World domesticates also have microcenter patterns in Asia Minor. This phenomenon is reported in more detail elsewhere (Harlan, 1951).

It is evident, therefore, that the microcenter variation pattern is not uncommon and may in fact be the rule within those broad geographic regions referred to by Vavilov as "centers of origin" or "centers of diversity." Many crops not only exhibit such discrete concentration patterns, but microcenters for many crops frequently coincide. Region 2 as indicated in figure 3 is the richest collecting grounds in Turkey since numerous crops exhibit a concentration of diversity in this area. The second richest collecting area is region 1.

With respect to the Vavilovian concepts of gene centers it is interesting to note that both regions 1 and 2 include an open plain. The Thracian wheat microcenter is so situated and can lay no claim to ecological diversity, remoteness from civilization, isolation, nor even to exceptionally primitive husbandry. Steel plows, grain drills, windmill-type binders are commonly used in Thrace. Agriculture here is as highly developed as anywhere in Turkey, but the unique wheat populations are maintained. Many other sections are agriculturally far more primitive and ecologically much more diverse and yet contain a comparative paucity of genetic variants.

TABLE 1

TURKISH CROPS REPRESENTED BY TRUE GENE MICROCENTER POPULATIONS OR BY EXCEPTIONAL CONCENTRATION OF VARIANTS IN SMALL GEOGRAPHIC AREAS

Species	Region (See fig. 3)
Amygdalus spp.	2, 3, 4, 5
Brassica napus	1
Cicer arietinum	1, 2, 4
Citrullus vulgaris	2 and central Anatolia
Cucumis melo	Two centers in region 1; one center in region 5
C. sativus	1, 2
Cucurbita moschata	2 and central Anatolia
C. pepo	2 and central Anatolia
Lens esculentum	1, 2, 3, 4
Lupinus spp.	1 and Mediterranean coast
Malus spp.	2, 3, 4, 5 and Black Sea coast
Medicago sativa	2, 4 and eastern Anatolia
Medicago spp. (annuals)	1 and Mediterranean coast
Onobrychis viceaefolia	2, 3, 4 and eastern Anatolia
Phaseolus vulgaris	1, 2, 3, 4 and Black Sea Coast
Pistacea spp.	2
Prunus spp.	2, 3, 4, 5 and Black Sea Coast
Pyrus spp.	2, 3, 4 and Black Sea Coast
Trifolium spp.	1 and Mediterranean Coast
Vicia faba	1, 2, 3, 4
Vicia spp. (vetches)	1 and Mediterranean Coast
Vitis vinifera	1, 2, 4
Zea mays	Black Sea Coast

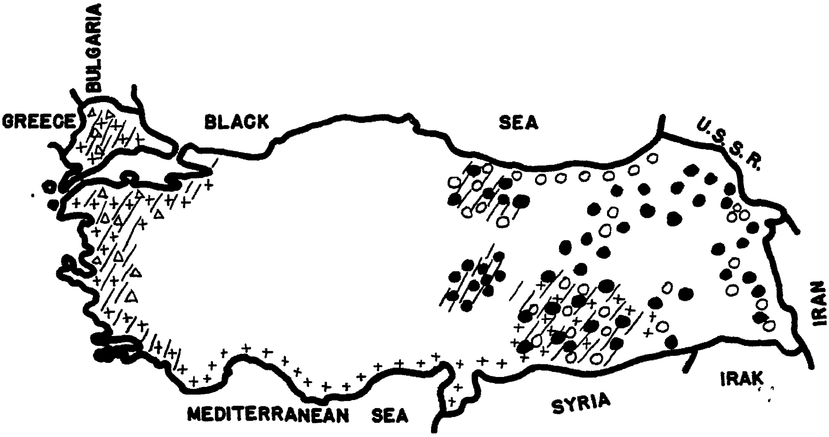

FIGURE 2. Additional centers of varietal diversity:

///// Leguminous grains
OoOo Fruit trees
+++ Annual forage legumes
●●● Perennial forage legumes
△△△ Rape

The nature of these microcenters should be studied by experimental attack. While an intensive survey of field populations in Turkey should yield the best results, a fairly adequate sampling of populations was brought to this country as a result of the expedition of 1948 and some of these materials are presumably available for study (Harlan, 1950).

The emphasis which Vavilov placed upon mountainous regions as the home of agriculture, upon mountain barriers as factors in isolation, upon remoteness from civilization and primitiveness of agriculture in the gene centers he described has probably led most agronomists to conclude that a gene center is a museum of archaic and primitive types which have been preserved in the isolation of hidden mountain valleys by the primitive husbandry of primitive people. This concept may have some application in some regions, but does not represent the whole story. Areas of varietal diversity may be found on the plains or in the mountains, remote from civilization or near to it. They may be very ancient or quite recent in development as indicated by the secondary centers of new world crops in Turkey. The fundamental characteristic which is common to all centers is that evolution is proceeding at a rapid pace *now*. As with all crop plants this evolutionary development is continually assisted or directed by the activity of man. No form of husbandry is too primitive or too specialized to have its effect upon the development of crop plants. Selections are made, natural hybrids occur; the very act of reaping and sowing favors one component of a population over another. New forms arise and are preserved or discarded or left to their fate under competition of a mixed population. It appears, therefore, that agronomists would be justified in accepting a more dynamic concept of a gene center

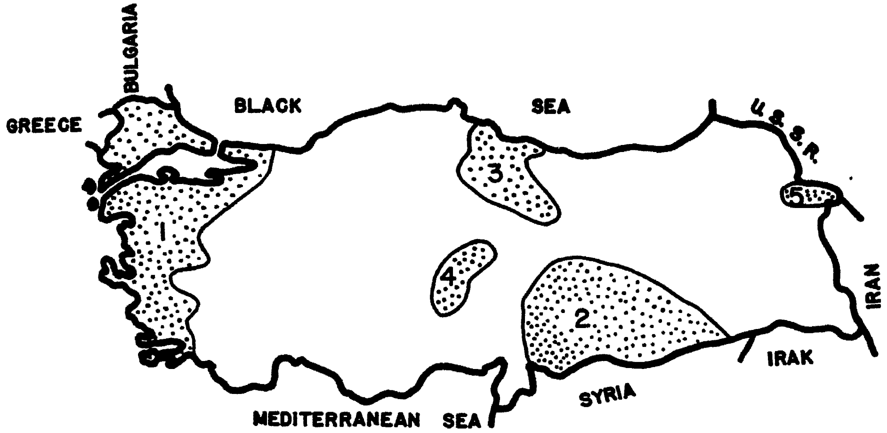

FIGURE 3. Areas of greatest crop diversity and varietal wealth:

Region 1. Wheats, muskmelons, rape, beans, lentils, chickpeas, broadbeans and annual forage legumes (vetches, lupines, clovers).
Region 2. Wheats, tree fruits, grapes, pumpkins, cushaws, watermelons, cucumbers, gourds, beans, lentils, chickpeas, broadbeans and both annual and perennial forage legumes.
Region 3. Fruit trees, beans, lentils, broadbeans and forage legumes.
Region 4. Fruit trees, grapes, beans, lentils, chickpeas, broadbeans and forage legumes.
Region 5. Fruit trees, muskmelons and forage legumes.

than Vavilov indicated, and that a center of origin as conceived by Vavilov may actually consist of an aggregate of small microcenters in which the present forms are continuously in the process of origination.

SUMMARY

Observations made by the author during an expedition to Asia Minor in 1948 led him to conclude that the geographic regions referred to by N. I. Vavilov as "centers of origin" or "gene centers" have a structure which is subject to study. Such geographic regions include areas of actual varietal paucity as well as small regions of enormous varietal wealth. The small areas in which is concentrated the varietal diversity of a crop are referred to as gene microcenters. Microcenters of a number of crops frequently coincide. Microcenters may be located on the plains or in mountainous regions, near civilization or remote from it; husbandry may be very primitive or moderately advanced. The one characteristic which is common to all microcenters is that evolution is proceeding at a rapid rate at the present time. An intensive survey of microcenter populations should yield immensely valuable information not only to the student of evolution but also to the agronomist and plant breeder.

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DUPLICATE GENES IN MAIZE

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Duplicate and triplicate factor ratios for various characters have long been known in tetraploid and hexaploid oats and wheat. Duplicate factors have also been reported in a number of known amphidiploids such as *Nicotiana tabacum* (Clausen and Cameron, 1950). The occurrence of 15:1 and 63:1 ratios in polyploid plants is presumptive evidence that some genes are in duplicate or triplicate and that identical or similar loci are present in the different genomes. The recent synthesis by McFadden and Sears (1946) of a vulgare wheat furnishes conclusive evidence that this hexaploid does consist of three different genomic sets of seven chromosomes each. Stadler's (1929) finding of a low induced mutation rate in tetraploid and hexaploid oats and wheat as compared to the much higher mutation rate in the diploid species likewise indicates a considerable duplication of loci in these plants. If 15:1 and 63:1 ratios are evidence of duplicated or triplicated loci in polyploids, do similar ratios in essentially diploid plants such as maize also indicate duplicated loci? That duplications played an important role in the evolution of the maize plant, and indeed in all organisms (Metz, 1947), can hardly be questioned, but it is possible that once homologous loci could become so differentiated by mutation that any trace of their former homology would disappear. Although Sprague (1932) has shown that 15:1 ratios cannot, at least for the inheritance of scutellum color in maize, be taken as critical evidence of duplicate genes, it appears likely that duplicate and triplicate factor inheritance is very suggestive of duplicated loci.

At least 14 cases of duplicate factor, two of triplicate and one of quadruplicate factor inheritance have been reported in maize (see Emerson, Beadle and Fraser, 1935). Unfortunately linkage determinations of duplicate loci are so arduous that nothing is known of the location of many of the sets. In eight of the 14 cases of duplicate factor inheritance the linkage relations of both loci are unknown while in three ($xn_1 xn_2$, $zg_1 zg_2$, $so_1 so_2$) one of the members of each set has been assigned to a specific chromosome, the other being unplaced. One of the genes of the $xn_1 xn_2$ set is in chromosome 10; in both the $zg_1 zg_2$ and $so_1 so_2$ sets one gene of each set has been located in chromosome 9 although the published data do not permit a precise determination of the map position. A rather surprising situation is found in the remaining three sets in that the $fr_1 fr_2$ loci are both in chromosome 7 (Jenkins and Pope in Emerson et al., 1935); the $w_5 w_6$ genes are both in chromosome 6 (Demerec, 1923); and the $au_1 au_2$ factors both lie in chromosome 9 (Eyster, 1929 and Emerson et al., 1935). Modified 15:1 ratios result when the duplicated loci are situated in the same chromosome, the observed ratio being a function of the recombination percentage between the two genes. According to Demerec the w_5 and w_6 genes lie approximately 22 recombination units on either side of the Y_1 locus. Eyster's data place au_1 about 11 units to the right of *Wx* in chromosome 9, while Jenkins and Pope's data locate fr_1 ten units to the right of *ij* and fr_2 15 units to the left of ra_1 and gl_1 in chromosome 7. If duplicate factor inheritance be accepted as indicative of the presence of duplications two conclusions can be drawn from the above data: (1) a disproportionately large number of the located duplicate factors lie in chromosome 9, and (2) inasmuch as a minimum of three of the 14 cases of duplicate factor inheritance have both loci situated in the same chromosome it appears that intra-chromosomal duplication was relatively more frequent than inter-chromosomal.

Maize may be an ancient amphidiploid and, if so, could have homologous segments in duplicate. Two relatives of maize, Coix and Sorghum, have species with a monoploid set of five chromosomes, and it is possible, as Edgar Anderson has recently emphasized, that maize originated from a cross of two five-chromosome species. The observation of occasional bivalent formation in monoploid plants is perhaps due to pairing between homologous segments in different chromosomes although chiasmata formation following non-homologous association might be responsible. Perhaps the most pertinent evidence for duplications is the disproportionate distribution of mutant genes in certain chromosomes, particularly chromosome 9. The long arm of 9 is twice the length of the short arm, yet a great majority of the thirty-odd mutants lie in the short arm. This non-random distribution has been interpreted as indicating the presence of redundant chromatin in the long arm (Anderson, 1938, Rhoades, 1945). It is possibly significant in this connection that four of the nine located duplicate loci are in chromosome 9 although in only one instance ($au_1\, au_2$ set) are the data sufficiently exact to place the au_1 gene in the long arm of 9.

A recent analysis of a new set of duplicate genes has disclosed evidence which is of some import to the above discussion. A viable pale green phenotype is produced when both pg_{11} and pg_{12} are homozygous recessive. The expected 15:1 ratios are found upon selfing $Pg_{11}\, pg_{11}\, Pg_{12}\, pg_{12}$ plants as are 3:1 ratios in F_2 populations if one locus is homozygous recessive and the second is heterozygous. The data in tables 1 and 2 place pg_{11} in chromosome 6 between the Y_1 and *Pl* loci. The summary of all linkage data gives an average recombination value of 21 per cent between Y_1 and pg_{11}. The pg_{12} locus in chromosome 9 has a mean recombination percentage of 10 with the *Wx* locus. The data in table 3 show that the linear order is *Sh*-*Wx*-Pg_{12}. According to Anderson and Randolph (1945) the centromere of chromosome 9 is not much more than two recombination units to the right of *Wx*. It follows, therefore, that the pg_{12} gene lies in the long arm of chromosome 9. Eyster's data placed au_1 about 11 units to the right of *Wx*. Even though recombination values are subject to considerable variation it seems highly probable that the pg_{12} and au_1 genes, both members of duplicate sets, lie close to one another in the long arm of chromosome 9—i.e., the arm which was suspected

TABLE 1

BACKCROSS DATA FROM CROSS OF Y_1-Pg_{11}-Pl/y_1-pg_{11}-pl Pg_{12} pg_{12} INDIVIDUALS BY y_1-pg_{11}-pl pg_{12} POLLEN. THE Y_1 AND Pl LOCI GIVE 1:1 RATIOS BUT THE PALE GREEN CHARACTER APPEARS IN A 3:1 RATIO SINCE DUPLICATE GENES ARE INVOLVED IN ITS EXPRESSION.

Y	*y*	*Y*	*y*	*Y*	*y*	*Y*	*y*	
Pg	*pg*	*pg*	*Pg*	*Pg*	pg	*pg*	*Pg*	
Pl	*pl*	*pl*	*Pl*	*pl*	*Pl*	*Pl*	*pl*	Σ
661	319	92	176	178	40	3	337	1806

Y_1-Pg_{11} = 20.9% recombination
Pg_{11}-Pl = 9.5% recombination
linear order is Y_1-Pg_{11}-Pl

of possessing redundant chromatin. Demerec's data place either w_5 or w_6 22 recombination units to the right of Y_1 in chromosome 6, which is nearly the same map position found for the pg_{11} gene. It can hardly be fortuitous that the au_1 and pg_{12} genes lie in the same region of chromosome 9 and that the w_5 or w_6 and pg_{11} genes are close together in chromosome 6. It appears probable that the long arm of 9 has a duplicated segment which includes the au_1 and pg_{12} loci, and that there is a duplication in the long arm of chromosome 6 between *Y* and *Pl* which carries either w_5 or w_6 and pg_{11}. It might be predicted that nests of duplicate loci will be found in both of these chromosomes when now-unplaced duplicate sets are involved in appropriate linkage tests, although it by no means follows that members of duplicate sets will be restricted to these two chromosomes.

Although the available evidence shows that the au_1 and pg_{12} genes are located in the long arm of chromosome 9 it does not follow that this arm is composed solely of redundant chromatin. Spores deficient for segments of the long arm of 9 are produced by adjacent segregations in plants heterozygous for reciprocal translocations. These deficient spores abort, hence

TABLE 2

BACKCROSS DATA FROM CROSS OF Y_1-Pg_{11}-Pl/y_1-pg_{11}-pl pg_{12} pg_{12} INDIVIDUALS BY y_1-pg_{11}-pl pg_{12} POLLEN. THE pg_{12} LOCUS IS HOMOZYGOUS RECESSIVE SO A 1:1 RATIO IS OBTAINED. THE POORER VIABILITY OF THE PALE GREEN PLANTS ACCOUNTS FOR THE DEFICIENCY OF THE PALE GREEN PHENOTYPE.

(0)	(0)	(1)	(1)	(2)	(2)	(1-2)	(1-2)	
Y	*y*	*Y*	*y*	*Y*	*y*	*Y*	*y*	
Pg	*pg*	*pg*	*Pg*	*Pg*	*pg*	*pg*	*Pg*	
Pl	*pl*	*pl*	*Pl*	*pl*	*Pl*	*Pl*	*pl*	Σ
128	93	32	23	27	11	3	1	318

Y_1-Pg_{11} = 18.6% recombination
Pg_{11}-Pl = 13.2% recombination
linear order is Y_1-Pg_{11}-Pl

TABLE 3

F$_2$ DATA FROM SELF-POLLINATION OF $\frac{Y_1\,Pg_{11}}{y_1\,pg_{11}}$ $\frac{Sb\,Wx\,Pg_{12}}{sb\,wx\,pg_{12}}$ PLANTS.

The Y_1 locus in chromosome 6 is independent of the *Sb* and *Wx* loci in chromosome 9 but Y_1, *Sb* and *Wx* all show linkage with the pale green character since it is due to duplicate genes, one of them being in chromosome 6 and the second in chromosome 9.

Y	*Y*	*Y*	*Y*	*Y*	*Y*	*Y*	*Y*	*y*	*y*	*y*	*y*	*y*	*y*	*y*	*y*	
Sb	*Sb*	*sb*	*sb*	*Sb*	*Sb*	*sb*	*sb*	*Sb*	*Sb*	*sb*	*sb*	*Sb*	*Sb*	*sb*	*sb*	
Wx	*Wx*	*Wx*	*Wx*	*wx*	*wx*	*wx*	*wx*	*Wx*	*Wx*	*Wx*	*Wx*	*wx*	*wx*	*wx*	*wx*	
Pg	*pg*	*Pg*	*pg*	*Pg*	*pg*	*Pg*	*pg*	*Pg*	*pg*	*Pg*	*pg*	*Pg*	*pg*	*Pg*	*pg*	
810	6	119	0	87	13	123	17	252	7	30	2	17	18	23	28	$\Sigma = 1552$

Y_1–Pg_{11} = 22% recombination
Sb–Pg_{12} = 25% recombination
Sb–Wx = 22% recombination
Wx–Pg_{12} = 7% recombination
Y_1–Sb = 50% recombination
Y_1–Wx = 48% recombination
linear order is Sb–Wx–Pg_{12}

this arm carries loci essential for normal gametophyte development which are not present in duplicate in other chromosomes of the haploid complement. Perhaps both the failure to find more mutant genes in the long arm and the abortion of deficient spores can be explained by intra-chromosomal duplications but this is highly speculative.

The data of table 3 are unusual in one respect. The *Y* locus in chromosome 6 shows 22 percent recombination with the pale green character and the *Wx* locus in chromosome 9 has seven percent recombination with the same character yet the *Y* and *Wx* loci are independent of one another. This situation is, of course, wholly explicable since the pale green phenotype depends upon duplicate factors. The linkages of *Y* and *Wx* with the same pale green character are due to the fact that each is linked with one of the two members of a set of duplicate genes.

Although it may be difficult to differentiate between them in practice a fundamental distinction exists between a suppressor and a duplicate gene. As its name signifies a duplicate gene is similar or identical to another locus; the recessive phenotype is not expressed unless the recessive alleles of both loci are homozygous. A suppressor gene inhibits or nullifies the effect of another, non-homologous locus. Houlahan and Mitchell (1947) were able to discriminate between a suppressor and a duplicate gene in Neurospora. In their study of mutations which control the synthesis of pyrimidine they found three different alleles at one locus. A mutation occurred which suppressed the effect of the three alleles—i.e., strains with both the suppressor mutant and a pyrimidineless gene had a normal growth habit on medium lacking pyrimidine. They believe that the suppressor mutant is not a duplicate of the wild type allele of the pyrimidine locus since strains lacking the suppressor but carrying the wild type allele grew normally in the presence of arginine while strains with the suppressor mutant and the pyrimidineless allele were inhibited.

✓ Lindstrom (1921) found that neither the L_1 nor the l_1 alleles had any effect on chlorophyll synthesis in maize. Their action was manifest only in the presence of other mutant genes. For example, seedlings homozygous for the white seedling gene w_1 were cream-white if the dominant L_1 allele was present but were yellow if the recessive l_1 allele was homozygous. The action of l_1 was not restricted to a specific w gene but modified the effect of a number of white and virescent genes. While nothing is known of the way in which the l_1 gene acts in producing a yellow rather than a white phenotype it may be considered as a partial suppressor of a large number of mutant loci. This behavior is in striking contrast to the specific action of the suppressor genes of Drosophila.

An unusual situation has been reported by Everett (1949) in maize. The recessive gene cl_1 produces seeds with light colored endosperm which when germinated give rise to albino seedlings. Everett considers cl_1 to be a pleiotropic gene. The Cl_2 allele, which segregates independently of cl_1, modifies the chlorophyll effect of cl_1 but has no effect on endosperm color. Seedlings of $cl_1\ cl_1\ cl_2\ cl_2$ constitution are albinos, those of $cl_1\ cl_1\ Cl_2\ cl_2$ are light green while those of $cl_1\ cl_1\ Cl_2\ Cl_2$ constitution are pseudo-normal. The pseudo-normal seedlings are green but die in the seedling stage as do the light green seedlings. The Cl_2 allele acts as a partial suppressor of cl_1. A third gene Cl_3 likewise has no effect on endosperm color of cl_1 kernels but $cl_1\ cl_1\ Cl_3\ cl_3$ and $cl_1\ cl_1\ Cl_3\ Cl_3$ plants are both green and viable. It was concluded that the Cl_3 gene is a semi-duplicate of the Cl_1 locus.

Aside from duplicate factor inheritance, further evidence of duplications in maize is Laughnan's (1949) recent demonstration that the A_1^b allele is composed of two separable components. His results are in some ways comparable to those found for the lozenge and Star-asteroid loci in Drosophila (Green and Green, 1949, Lewis, 1945) where, at least for Star-asteroid, there is cytological evidence of a duplicated locus. McClintock has interpreted (quoted in Metz, 1947) her data on pseudo-alleles near the end of the short arm of chromosome 9 of maize as indicating the presence of a series of similar or identical genes which have arisen through duplication of individual loci or short segments.

That the architecture of the germ plasm of maize contains duplicated regions can hardly be doubted but whether or not they represent vestiges reflecting an ancient amphidiploid origin or represent later occurring duplications cannot be decided at this time.

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METABOLIC TYPES AND GROWTH TYPES[1]

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The problem of the relation between metabolism and body size is one of the classical topics of physiology. It is usually treated in terms of the surface rule, established by Rubner in 1883, and heralded by earlier work of Sarrus and Rameaux, Bergmann and Richet. The surface rule states that the metabolic rate per unit weight decreases with increasing size, but is constant per unit surface. Rubner explained the surface rule in terms of homeothermy: Since all warm-blooded animals heat their bodies to a temperature of approximately 37°C, and since heat output takes place on the body surface, the same number of calories must be produced per unit surface. Even in recent discussions (Brody, 1945; Kleiber, 1947; Krebs, 1950) homeothermic animals are taken almost solely into consideration. It is necessary, however, to consider the problem on the broader basis of comparative physiology.

Such investigation has been carried through by the author and his co-workers (Bertalanffy, 1942 et seq.). The main results of this re-examination of the problem are:

(1) Recent investigation shows that the surface rule also holds for poikilothermic vertebrates and certain invertebrates. The rule as such is of a wide application, but current explanations, especially the explanation based on homeothermy, are too restricted.

(2) On the other hand, there are many classes of animals for which the surface rule does not apply.

(3) Thus we came to the statement of several *metabolic types* in respect to the relation between metabolic rate and body size. So far, three metabolic types have been distinguished.

In the first type, metabolic rate is proportional to a surface or to the ⅔ power of weight. Representatives of this type are fish, but also certain invertebrates, such as isopod crustaceans, according to our own investigations (Bertalanffy and Müller, 1943c), mussels (Weinland, 1919; Ludwig and Krywienczyk, 1950), and Ascaris (Krüger, 1940). As an example, metabolic rates in the sow bug, Armadillidium, are presented in table 1. The rate of oxygen consumption decreases per unit weight, but is constant per unit surface, expressed as the ⅔ power of weight. The most important question is, of course, whether the surface rule applies to mammals. This

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TABLE 1

CO$_2$ PRODUCTION OF ARMADILLIDIUM PALLASII

(21°, mean values of 55 determinations)

weight in mg	15	33	50	100	160
cmm O$_2$/h	3,0	5,2	7,2	11,2	15,2
per gm/h	200	174	144	112	94
per unit surface ($W^{2/3}$)/h	48,5	54,2	53,0	49,8	51,6

question has been much discussed in recent years (Brody, 1945; Kleiber, 1947). In our work, the surface rule appears to be valid in the rat, comparing metabolic rates in individuals of different size (unpublished).

The second type is entirely different. Here metabolic rate is directly proportional to weight. The most important cases are insect larvae (Bertalanffy and Müller, 1943a; Teissier, 1931), and insects in interspecific comparison (Kittel, 1941), but equally hemimetabolic insects (Bertalanffy and Müller, 1943b) belong to this type. The example given is the walking stick, *Dixippus morosus.* Table 2 shows the constancy of metabolic rates per unit weight. Also land snails of the order Helicidae (intraspecific comparison: Bertalanffy and Müller, 1943a; interspecific comparison: Liebsch, 1929), and annelids, such as the earth worm (Bertalanffy and Müller, 1943c) belong to this type.

TABLE 2

OXYGEN CONSUMPTION OF DIXIPPUS MOROSUS

(20°, mean values of 20 determinations)

weight in mg	8	130	250	450	630	850
cmm O$_2$/h	2,0	30,6	60,7	113,2	154,8	206,6
per gm/h	250	236	243	252	245	242

In the third type, metabolic rate is intermediate between weight proportionality and proportionality to surface. To this type belong pond snails such as Planorbis and Limnaea, and Planarians (Bertalanffy and Müller, 1943a). Table 3 demonstrates that metabolic rates decrease with respect to weight, but increase with respect to surface.

Now just as there are different types of metabolism, there are also different types of growth. The most common type of animal growth is that growth rates continually decrease with time and finally the organism reaches

TABLE 3

OXYGEN CONSUMPTION OF PLANORBIS SPEC.

(23°, mean values of 48 determinations)

weight in mg	30–35	58–62	90–100	140	190–200
cmm O$_2$/h	2,3	3,9	5,4	7,3	9,5
per gm/h	69	65	56	52	48
per unit surface ($W^{2/3}$)/h	22,9	25,1	26,1	27,0	28,2

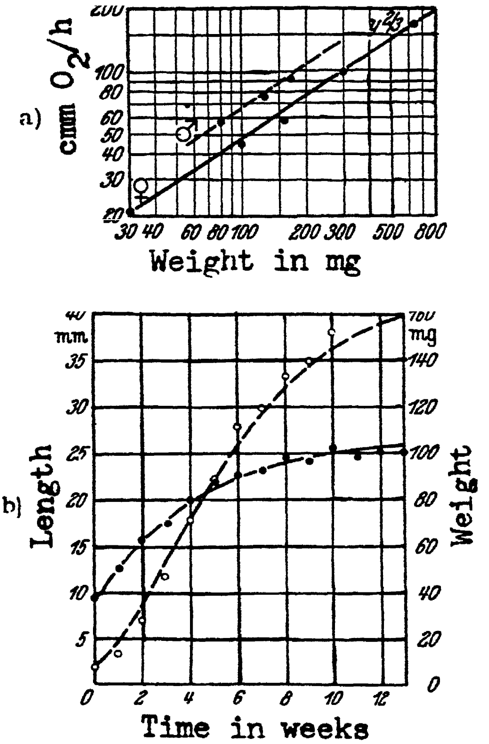

FIGURE 1. (a) Respiration at 20°C (mean values of 85 determinations); (b) growth curves in the fish, *Lebistes reticulatus*. Calculation of linear growth: ————; of weight growth: — — — — —. Equation for linear growth:

$$x = X - (X - x_0)e^{-kt};$$

for weight growth:

$$y = \left[\sqrt[3]{Y} - (\sqrt[3]{Y} - \sqrt[3]{y_0})\right] e^{-kt}.$$

x, y = length, weight at time t; X, x_0, Y, y_0 = final and initial length and weight, respectively; $k = K/3$.

a steady state in the adult. There are, however, also other types of animal growth. It appears that it is possible to establish a *strict connection between growth types and metabolic types* with respect to the dependence of metabolic rate on body size. We give typical examples for these types.

In the first type, metabolism is surface-proportional. Figure 1 shows metabolism and growth in the guppy, *Lebistes reticulatus*. Metabolic rates are presented in allometric or log-log plot against weight. In the case of surface proportionality, the allometric line has a slope of ⅔. The corresponding growth curve of this type is characterized as follows: (1) Growth rates are decreasing and the growth process attains a steady state; (2) the curves of growth by weight and linear growth show characteristic differences: the curve of weight growth has a point of inflection at about ⅓ of the final weight, the curve of linear growth shows simple exponential decrease.

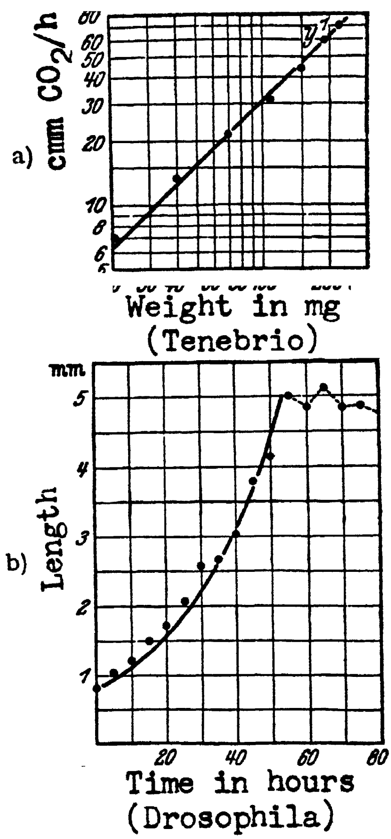

FIGURE 2. (a) Respiration at 20°C (mean values of 84 determinations); (b) growth (data of Alpatov) in insect larvae. Growth equations:

$$x = x_0\, e^{ct};\ y = y_0\, e^{3ct}.$$

x, y = length, weight at time t; x_0, y_0 = initial length, weight; c = constant.

As an example of the second type, we give metabolism and growth in insect larvae (figure 2). In this type, metabolic rate is weight-proportional and therefore gives an allometric line of slope 1. Growth is here exponential, that is, growth rates always increase and no steady state is reached. Growth is only intercepted by a sort of crisis which is represented by metamorphosis in insect larvae, by seasonal cycles in land snails, which, as said before, also belong to this type.

In the third type, metabolic rate stands between proportionality to weight and proportionality to surface, giving an allometric line of a slope between ⅔ and 1. Our example is the pond snail, Planorbis (figure 3). In this case the curve of weight growth does not differ much from that of the first type, but the curve of linear growth is characteristic. While in the first type the curve has no point of inflection, it has one in the third type.

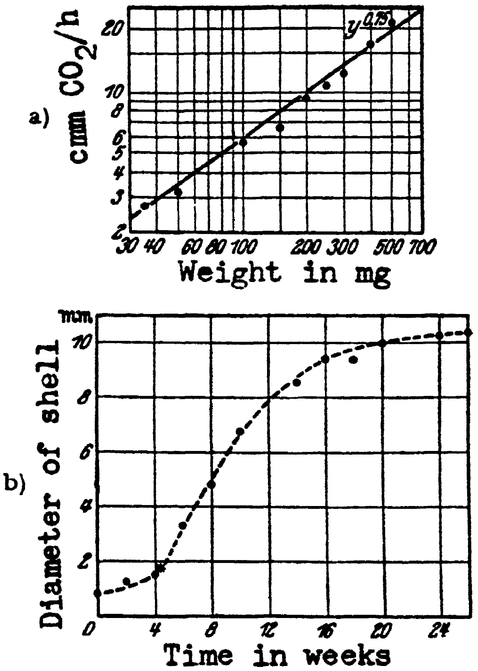

FIGURE 3. (a) Respiration at 23°C (mean values of 67 determinations); (b) growth in *Planorbis spec.* The theoretical point of inflection is indicated by an asterisk (*).

The relation between metabolic types and growth types can be explained, and many surprising predictions can be made on the basis of a theory on animal growth advocated by the author (1941, 1942, 1948, 1949). Growth is considered to be the result of a counteraction of anabolism and catabolism of building materials, according to the following basic expression:

$$\frac{dy}{dt} = \eta y^m - \kappa y^n.$$

In words: the change of body weight y is given by the difference between the processes of building up and breaking down: η and κ are constants of anabolism and catabolism respectively, while the exponents m and n indicate that the latter are proportional to some powers of body weight y. Catabolism is, at least in a first approximation, proportional to weight so that the exponent m can be set equal to 1. Inserting for the exponent of anabolism m that value which is found for size dependence of metabolism, the types of growth automatically follow and the formulas for laws governing the several growth types can be deduced. The mathematical formulations are given elsewhere (Bertalanffy, 1941, 1942). It can be said, however, that these deductions have been found valid in all cases sufficiently investigated. Ludwig (1950) who is one of the most active workers in this field in Continental Europe, states that "no contradictions were found in his experiments to Bertalanffy's theory."

The relations found are summarized in table 4, which indicates the metabolic types, the corresponding growth types, and examples investigated. It is hoped that these investigations will lead to a comparative physiology of metabolism and growth.

TABLE 4

METABOLIC TYPES AND GROWTH TYPES

Metabolic type	Growth type	Examples
I. Respiration *surface-proportional*	*(a)* Linear growth curve: attaining *without inflexion* a steady state. (b) Weight growth curve: *sigmoid,* attaining, with inflexion at c. ⅓ of final weight, a steady state	Lamellibranchs, fish, mammals
II. Respiration *weight-proportional*	Linear and weight growth curves *exponential,* no steady state attained, but growth intercepted by metamorphosis or seasonal cycles	Insect larvae, Orthoptera, Helicidae
III. Respiration *intermediate between surface- and weight-proportionality*	(a) Linear growth curve: attaining *with inflexion* a steady state. (b) Weight growth curve: *sigmoid,* similar to I(b)	Planorbidae

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A SURVEY OF CHEMICALS FOR MUTAGENIC ACTION ON E. COLI[1]

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INTRODUCTION

At present we have available two good methods, using *Escherichia coli,* for the quantitative study of spontaneous mutability and of the mutagenic action of various agents such as radiations and chemicals. The older of the two methods deals with mutational changes from phage sensitivity to phage resistance, and the more recently developed one (Demerec, 1951; Bertani, 1951) with back-mutations from streptomycin dependence to nondependence. In both cases the mutants can be easily classified; very large numbers of bacteria may be tested; and slight increases in mutation rate can be easily detected.

After the streptomycin method had been developed we undertook extensive tests of a number of chemicals for mutagenicity, using mainly this method because it is the simpler of the two.

SELECTION OF CHEMICALS

Previous work by Witkin with *E. coli* (Witkin, 1947; Demerec, Wallace, and Witkin, 1948) had indicated that compounds exhibiting quite different properties may all be mutagenic. Using the phage method, she found that sodium desoxycholate, pyronin, acriflavine, caffeine, and colchicine acted as mutagens, but detected no mutagenic effect in tests with methyl green, basic fuchsin, and methylcholanthrene endosuccinate.

In planning the present survey, therefore, we prepared a list of chemicals, including representatives of widely separated groups, from simple inorganic and organic compounds to complex organic chemicals. Before the scheduled tests had been completed, however, we found that certain ferrous and manganous salts are very potent mutagens, and our attention was thus diverted to a detailed study of these compounds. Since it will require some time to carry this out, the data so far available from the survey are being summarized here.

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MATERIALS AND METHODS

The "streptomycin" method for detection of induced mutations has been fully described by Bertani (1951). Only technical details will be given here.

Strains B/Sd-4/1,3,4,5 and B/Sd-4/3,4 of *E. Coli* were used throughout this work. For every experiment, bacteria were grown for 24 hours at 37°C in an aerated broth culture containing 10 micrograms of streptomycin per milliliter. They reached a saturation titer of approximately 2 to 3×10^9 cells per milliliter. Each culture was started from an inoculum, usually large, taken from streptomycin-agar slants kept in a refrigerator. Before treatment the bacteria were washed in saline and resuspended in distilled water. A sample of the new suspension was added to the desired solution of chemical in distilled water, and incubated at 37°C for a certain period of time; no growth occurs under these conditions. Another sample of the same suspension was added to an equal amount of distilled water and incubated for the same period of time, as a control. At the end of the treatment period, both treated and control suspensions were assayed by plating suitable dilutions on streptomycin-agar plates. At the same time they were plated (0.1 ml per Petri dish), either undiluted or diluted not more than 1:10 in plain broth, onto a number of streptomycin-free plates, using a glass spreader and a turntable. The assay plates were incubated for 48 hours, after which it was possible to count the colonies and calculate the titers of the two suspensions at the end of treatment, and the percentage of survivors. The streptomycin-free plates (mutant plates) were incubated for at least six days. After this time the colonies were scored, and the frequency of mutants calculated by dividing the number of colonies by the number of (viable) bacteria plated.

The length of the period of incubation in distilled water does not appreciably affect the proportion of mutations in the controls. In all but a few exceptional cases, nevertheless, the control suspensions were incubated for the same period of time as the chemically treated suspensions.

The degree of crowding of the dependent bacteria on the mutant plates would be expected to affect the observed frequency of mutants, as it influences the extent of residual growth and thus the chance that a mutant cell will develop into a visible colony. This effect is slight, however, when the bacteria plated have been raised in 10 micrograms of streptomycin per milliliter.

The chemical treatment also may limit the extent of residual growth, causing a spurious lowering of the frequency of mutants in the treated series. This may explain why, in experiments with some chemicals, the treated series showed frequencies lower than those of the controls.

Several of the chemicals used cause the formation of clumps of bacteria during treatment. If these clumps have not dissolved by the time the treated suspension is assayed to determine survival, the observed survival value will be too low, and consequently the calculated mutation frequency too high. Therefore, except in the very first experiments done (*i.e.*, those with sulfuric, nitric, and hydrochloric acids, which in any case were not found to be mutagenic), the treated suspensions were always examined under the microscope before plating, and discarded if clumping was detected. By varying the concentration of chemical and the length of treatment, it was possible to eliminate clumping in many cases.

In a few scattered experiments (and in most of those done with phenol and formaldehyde) the dependent bacteria were grown in 25 instead of 10 micrograms of streptomycin per milliliter. It is known that this results in greater residual growth and therefore a larger number of spontaneous mutants per plate. This explains why in some experiments the spontaneous mutant frequencies were higher than average.

RESULTS

The results of a number of experiments for each chemical are reported in table 1. The concentration of the chemical shown in column 2 is the effective concentration at the time of treatment (*i.e.*, corrected for the addition of the bacterial suspension in distilled water). Number of plates (column 5) is the number of streptomycin-free plates used for the scoring of mutants. Total number of bacteria (column 6) is the number of viable dependent bacteria put on each plate, multiplied by the number of plates used. Number of mutants per 10^8 bacteria (column 7) is the mutant frequency, calculated by dividing the total number of colonies scored in an experiment by the total number of bacteria. The total number of mutant colonies is not given in the table, but can easily be calculated by the inverse operation. From it, one can determine the average number of mutants per plate, which in the experiments reported here was rarely larger than 10–20. Assuming a Poisson distribution, the variance can be estimated and the statistical significance of the results evaluated. Sometimes the same controls were common to several treated series; and in these cases the number of plates and total number of bacteria are reported only once for the controls. Different experiments done with each chemical are listed in order of decreasing survival.

Inorganic acids. Tests made with sulfuric, orthophosphoric, nitric, and hydrochloric acids gave negative results. Under certain conditions, all four of these compounds produced clumping of the treated bacteria, and this explains why in some experiments the values obtained for mutant frequency were higher in the treated series than in the controls. Negative results for hydrochloric acid had also been obtained in earlier experiments with Drosophila (Goldat and Beliaieva, 1935).

Boric acid was the only inorganic acid tested that gave positive results. In all experiments but one, the treated series showed an increase in mutation frequency. No clumping was ever observed among bacteria treated with boric acid.

Alkalies. Sodium hydroxide and potassium hydroxide were tested, with inconclusive results. Under the conditions of these experiments they never produced clumping.

Ammonia. Ammonia had been used in experiments with Drosophila by Lobashov and Smirnov (1934) and Lobashov (1937), and found to have mutagenic action. In the tests reported here, ammonia showed definite mutagenic activity in experiments where the proportion of survivors was lower than 2 per cent. Apparently similar positive results were obtained with ammonium chloride; they are not reported here, however, because clumping could not be avoided after treatment with this chemical.

Hydrogen peroxide. Attention was first drawn to the mutagenic activity of peroxides by the work of Stone, Wyss, and Haas (1947) on the indirect effect of mutation-inducing radiations on bacteria. Dickey, Cleland, and Lotz (1949), using Neurospora, studied the mutagenic potency of hydrogen peroxide and of a number of organic peroxides, and obtained positive results. In our experiments also, hydrogen peroxide was clearly shown to be mutagenic.

Metal salts. Mercuric chloride, silver nitrate, copper sulfate, and sodium silicate were tested. Only copper sulfate showed indications of mutagenic effect in the lower range of survival. Mutagenic potency had already been demonstrated for this salt by Magrzhikovskaja (1936) and Law (1938) in experiments with Drosophila. The results reported for mercuric chloride and silver nitrate do not exclude the possibility of their having mutagenic activity at the lowest survival levels, because it was extremely difficult to obtain survivals below 1.0 per cent and yet not so low as to fall beyond the range of sensitivity of the method.

Organic acids. Lobashov and Smirnov (1934) and Lobashov (1937) had treated Drosophila with acetic acid, but failed to detect any mutagenic activity of this substance. In our experiments, acetic, formic, and lactic acids were tested. The first two showed mutagenic activity. Some of the experiments with lactic acid indicated a weak mutagenic effect; but this is rendered doubtful by the fact that experiments with sodium lactate consistently gave negative results.

Formaldehyde. The mutagenic potency of this substance had been discovered by Rapoport (1946) and confirmed by W. D. Kaplan (1948) and Auerbach (1949), working with Drosophila. Clear-cut positive results were obtained in our experiments also. Auerbach's hypothesis that production of mutations by formaldehyde can occur only through some intermediate effect on substances contained in Drosophila food is not supported by our experiments, in which the treatment was carried out on washed bacteria.

Phenol. The mutagenic action of phenol had been discovered by Hadorn and Niggli (1946), working on Drosophila with a very special treatment technique. Other techniques had failed to give positive results. Negative results had been obtained by Dickey, Cleland, and Lotz (1949) with Neurospora. Our tests gave clear-cut positive results for this substance, and also for two closely related substances, alpha-dinitrophenol and trinitro-

TABLE 1

RESULTS OF EXPERIMENTS TESTING INDUCTION OF BACK-MUTATIONS IN Sd-4 (STREPTOMYCIN-DEPENDENT) *E. COLI* TREATED WITH CERTAIN CHEMICALS

Chemical	Concentration %	Treatment (hours)	Survival %	Treated			Control		
				No. of plates	Total no. of bacteria	Mutants per 10^{8} bacteria	No. of plates	Total no. of bacteria	Mutants per 10^{8} bacteria
Sulfuric acid	0.003	3	13.	10	1.2×10^{8}	11.8	4	8.5×10^{7}	8.3
	0.002	3	12.	8	8.0×10^{7}	70.0	4	7.0×10^{7}	147.
	0.002	3	11.	8	1.1×10^{8}	28.7	4	8.4×10^{7}	35.4
	0.005	3	7.0	9	4.6×10^{7}	10.9	4	5.4×10^{7}	33.1
	0.002	3	1.9	8	2.2×10^{7}	74.	4	9.2×10^{7}	98.1
Phosphoric acid (ortho-)	0.008	3	29.	8	1.9×10^{8}	29.4	4	6.4×10^{7}	35.9
	0.008	3	17.	8	1.0×10^{8}	16.5	4	7.3×10^{7}	12.3
	0.010	3	1.2	8	9.3×10^{7}	2.2	4	8.9×10^{7}	21.3
Nitric acid	0.008	3	8.8	10	8.1×10^{7}	11.1	4	8.5×10^{7}	8.3
	0.005	3	8.3	8	5.8×10^{7}	90.2	4	7.0×10^{7}	147.
	0.005	3	6.9	8	6.8×10^{7}	16.2	4	8.4×10^{7}	35.4
	0.006	3	3.5	10	2.6×10^{7}	11.7	4	5.4×10^{7}	33.1
	0.005	3	2.7	8	2.2×10^{7}	111.1	4	9.2×10^{7}	98.1
Hydrochloric acid	0.0015	3	100.	5	6.0×10^{7}	6.7	4	4.8×10^{7}	10.4
	0.0015	3	33.	5	4.0×10^{8}	5.8	4	9.6×10^{7}	24.0
	0.00375	3	21.	5	2.5×10^{8}	6.8			24.0
	0.0075	3	11.	10	1.3×10^{7}	30.1			10.4
	0.00075	3	0.8	3	1.1×10^{7}	0.0	2	9.2×10^{7}	3.6
	0.0015	3	0.2	4	4.0×10^{6}	0.0			3.6
	0.0015	3	0.2	4	3.8×10^{6}	25.0	5	2.5×10^{8}	26.2
Boric acid	4.0	3	50.	8	1.0×10^{9}	27.3	6	7.6×10^{7}	6.6
	1.8	24	33.	8	6.0×10^{8}	28.9	5	1.1×10^{8}	7.9
	2.0	24	25.	8	4.6×10^{8}	28.7			7.9
	2.0	24	24.	8	3.3×10^{8}	82.3	6	1.0×10^{8}	5.8

TABLE 1—*continued*

Chemical	Concentration %	Treatment (hours)	Survival %	Treated			Control		
				No. of plates	Total no. of bacteria	Mutants per 10^8 bacteria	No. of plates	Total no. of bacteria	Mutants per 10^8 bacteria
Boric acid *(continued)*	2.0	24	17.	8	1.1×10^8	6.6	6	4.6×10^8	9.8
	2.0	24	11.	8	1.6×10^8	64.1	6	1.0×10^8	13.6
	1.5	24	11.	8	8.3×10^7	31.2	4	8.9×10^7	21.3
	1.7	24	10.	9	5.9×10^7	20.3	6	9.0×10^7	6.7
	2.0	48	9.7	8	9.2×10^7	16.4	4	7.0×10^7	5.7
	1.9	24	3.3	10	2.2×10^7	18.6			6.7
	1.8	24	2.8	8	2.1×10^7	56.6	4	7.5×10^7	30.6
Sodium hydroxide	0.013	3	86.	10	2.6×10^9	11.7	5	3.0×10^8	18.9
	0.015	3	37.	10	3.0×10^8	32.4	4	6.4×10^7	35.9
	0.02	3	27.	7	5.4×10^8	13.8	5	1.4×10^8	16.8
	0.011	3	24.	10	3.4×10^8	3.3	6	1.9×10^8	3.6
	0.0145	3	20.	10	1.9×10^8	20.2	4	8.5×10^7	8.3
	0.015	3	16.	10	1.2×10^8	16.3	4	7.3×10^7	12.3
	0.0125	3	12.	8	1.2×10^8	28.2	4	1.0×10^8	38.0
	0.0125	3	11.	8	1.0×10^8	130.7	4	9.2×10^7	98.1
	0.014	3	5.7	8	5.3×10^7	43.0	4	6.8×10^7	43.8
	0.022	3	1.3	8	2.3×10^7	21.6	5	1.1×10^8	12.1
Potassium hydroxide	0.00945	3	44.	10	2.8×10^8	10.3	6	9.0×10^7	6.7
	0.015	3	42.	8	3.8×10^8	55.5	4	9.2×10^7	98.1
	0.016	3	32.	8	3.0×10^8	43.0	4	6.8×10^7	43.8
	0.015	3	29.	8	2.0×10^8	79.2	4	7.0×10^7	147.
	0.019	3	20.	10	3.6×10^8	12.5	6	1.8×10^8	5.5
	0.018	3	18.	10	1.4×10^8	9.9	6	1.1×10^8	9.7
	0.018	3	19.	10	3.4×10^8	20.1			5.5
	0.0185	3	13.	10	1.9×10^8	6.4	6	1.9×10^8	3.6
	0.0165	3	13.	10	1.8×10^8	4.4			3.6
	0.017	3	10.	10	7.9×10^7	12.7			9.7

TABLE 1 (*continued*)

Chemical	Concentration %	Treatment (hours)	Survival %	Treated			Control		
				No. of plates	Total no. of bacteria	Mutants per 10^8 bacteria	No. of plates	Total no. of bacteria	Mutants per 10^8 bacteria
Potassium hydroxide (*continued*)	0.019	24	8.5	10	1.5×10^8	17.8			5.5
	0.018	24	8.3	10	1.5×10^8	9.4			5.5
	0.0175	3	2.9	8	2.9×10^7	37.6	4	1.0×10^8	38.0
Ammonia	0.025	3	66.	6	2.4×10^9	4.9	2	1.8×10^9	6.8
	0.100	3	26.	9	1.1×10^8	2.6	5	2.5×10^8	3.7
	0.250	3	13.	9	3.9×10^8	10.3	5	1.7×10^8	10.0
	0.250	3	12.	8	3.1×10^8	1.6	4	1.4×10^9	2.8
	0.300	3	9.6	5	2.4×10^8	3.4	5	2.5×10^9	2.6
	0.400	3	2.3	10	1.1×10^8	8.0			2.6
	0.250	3	1.2	8	2.4×10^7	37.5	5	1.3×10^9	12.9
	0.500	3	1.0	4	1.2×10^7	41.6	5	1.5×10^9	13.3
	0.500	3	0.4	10	2.0×10^7	121.			2.6
	0.500	3	0.2	10	5.6×10^6	90.			12.9
Hydrogen peroxide	0.150	4	78.	9	1.9×10^9	19.9	8	2.2×10^9	5.0
	0.195	4	59.	8	1.3×10^9	28.7			5.0
	0.195	½	45.	6	5.2×10^8	124.	8	1.5×10^9	6.9
	0.225	½	32.	8	4.9×10^8	121.			6.9
	0.195	½	3.1	8	5.4×10^7	175.	6	1.3×10^9	4.7
	0.240	4	1.4	9	3.5×10^7	120.			5.0
	0.195	½	0.6	8	1.1×10^7	26.7			4.7
Mercuric chloride	0.00005	6	61.	9	5.8×10^8	1.7	5	1.5×10^9	0.7
	0.00030	3	40.	10	4.1×10^8	4.2	4	4.1×10^8	12.3
	0.00025	3	21.	4	3.1×10^8	5.9	4	1.5×10^8	2.0
	0.00030	3	18.	3	2.5×10^8	2.9	2	9.2×10^7	3.3
	0.00005	9	11.	9	1.1×10^8	3.7			0.7
Silver nitrate	0.000005	24	84.	9	3.3×10^8	4.8	6	2.6×10^8	2.3
	0.000100	3	79.	10	1.6×10^8	2.5	5	1.0×10^8	8.0
	0.000050	3	75.	9	1.1×10^9	6.7	7	1.1×10^9	8.6

TABLE 1 (*continued*)

Chemical	Concentration %	Treatment (hours)	Survival %	Treated			Control		
				No. of plates	Total no. of bacteria	Mutants per 10^8 bacteria	No. of plates	Total no. of bacteria	Mutants per 10^8 bacteria
Silver nitrate (*continued*)	0.000025	3	59.	9	2.3×10^8	2.1			2.3
	0.000100	3	26.	9	3.8×10^8	7.4			8.6
	0.000100	24	4.3	9	6.1×10^7	8.2			8.6
Copper sulfate	0.00020	3	88.	9	1.4×10^9	5.7	6	1.0×10^9	5.1
	0.00040	3	34.	9	4.7×10^8	3.6	8	1.2×10^9	8.8
	0.00050	3	22.	10	5.0×10^8	4.4	5	1.1×10^9	8.9
	0.00050	3	14.	9	3.8×10^8	10.6	5	1.5×10^9	2.1
	0.00050	3	12.	3	1.1×10^8	20.7	4	1.2×10^8	20.8
	0.00075	3	2.4	9	3.7×10^7	40.5			5.1
	0.00100	3	0.36	9	7.2×10^6	55.5			8.9
	0.00125	3	0.22	10	4.9×10^6	0.0			8.9
Sodium silicate	0.025	3	66.	10	1.7×10^8	5.9	5	1.3×10^8	6.3
	0.100	3	33.	5	4.2×10^8	2.4	5	1.3×10^9	5.3
	0.050	3	27.	10	6.9×10^7	8.7			6.3
	0.100	3	16.	7	3.8×10^8	6.6	5	1.7×10^9	6.1
	0.100	3	4.6	9	7.9×10^7	11.4	7	1.3×10^9	6.2
	0.150	3	3.0	9	5.1×10^7	2.0			6.2
	0.300	3	0.11	5	1.3×10^6	0.0	5	1.2×10^9	7.0
Acetic acid	0.03	3	79.	10	1.3×10^9	8.3	5	1.6×10^8	10.0
	0.03	3	36.	8	9.9×10^8	36.4	5	1.7×10^8	9.2
	0.03	3	11.	10	8.5×10^7	15.3			10.0
	0.01	24	7.2	8	7.6×10^7	19.8	6	7.9×10^7	10.1
	0.03	3	6.0	10	9.5×10^7	25.2			10.0
	0.03	3½	0.9	8	8.8×10^6	56.8	6	7.6×10^7	6.6
Lactic acid	0.010	3	40.	10	6.4×10^8	12.8	5	1.6×10^8	10.0
	0.010	3	36.	10	2.9×10^8	10.7			10.0
	0.019	3	14.	8	2.0×10^8	16.7	5	8.9×10^7	4.5

TABLE 1 (*continued*)

Chemical	Concentration %	Treatment (hours)	Survival %	Treated			Control		
				No. of plates	Total no. of bacteria	Mutants per 10^8 bacteria	No. of plates	Total no. of bacteria	Mutants per 10^8 bacteria
Lactic acid (*continued*)	0.021	3	7.5	8	1.1×10^8	40.2			4.5
	0.020	3	6.8	8	1.7×10^8	17.0	5	1.6×10^8	15.3
	0.020	3	4.6	8	6.7×10^8	32.9	5	9.2×10^7	27.3
	0.020	4	2.5	8	2.9×10^7	34.7	6	8.5×10^7	9.4
	0.020	3	1.9	8	2.6×10^7	11.4	6	1.0×10^8	4.8
Sodium lactate	2.5	3	85.	8	1.5×10^9	7.3	5	1.1×10^8	12.1
	3.0	3	61.	8	1.7×10^9	16.4	5	1.6×10^8	15.3
	2.0	24	8.3	8	8.7×10^7	5.8	6	7.9×10^7	10.1
	2.5	24	1.4	8	3.8×10^7	5.2	5	1.7×10^8	9.2
Formic acid	0.0070	3	100.	8	1.5×10^9	18.1	6	1.1×10^8	2.7
	0.0060	3	93.	8	1.4×10^9	18.4			2.7
	0.0050	3	77.	8	1.3×10^9	20.2	5	1.1×10^8	12.1
	0.0065	3	30.	8	8.2×10^8	44.0	5	1.7×10^8	9.2
	0.0060	3	20.	8	5.0×10^8	43.3	5	1.6×10^8	15.3
	0.0065	3	19.	8	4.6×10^8	24.4	5	1.5×10^8	3.3
	0.0060	3	15.	8	1.4×10^8	27.7	6	7.1×10^7	2.8
	0.0075	3	3.4	8	8.2×10^7	38.0			3.3
	0.0070	3	2.8	8	2.6×10^7	38.5			2.8
Formaldehyde	0.01	3	61.	2	7.6×10^7	400.	2	1.3×10^8	26.2
	0.01	3	58.	5	6.8×10^8	133.	4	9.4×10^8	15.5
	0.02	3	58.	5	6.8×10^8	157.			15.5
	0.01	3	48.	4	3.7×10^7	245.	4	7.6×10^7	79.
	0.01	3	48.	4	3.7×10^6	470.	4	7.6×10^6	68.
	0.01	3	21.	8	1.3×10^8	120.	4	7.3×10^7	12.3
	0.02	3	16.	8	1.0×10^8	129.			12.3
	0.04	3	9.0	7	1.5×10^8	326.			15.5
	0.05	3	1.7	10	3.2×10^6	560.			79.

TABLE 1 (*continued*)

Chemical	Concentration %	Treatment (hours)	Survival %	Treated			Control		
				No. of plates	Total no. of bacteria	Mutants per 10^8 bacteria	No. of plates	Total no. of bacteria	Mutants per 10^8 bacteria
Phenol	0.10	3	90.	10	4.4×10^8	84.5	4	2.0×10^8	74.5
	0.10	3	69.	9	1.2×10^9	2.5	5	9.5×10^8	0.6
	0.08	3	48.	8	7.4×10^8	3.4			0.6
	0.20	3	43.	9	7.5×10^8	2.5			0.6
	0.10	12	1.7	10	7.3×10^6	124.	5	2.2×10^8	74.8
	0.10	24	1.1	10	3.1×10^6	192.	4	1.1×10^8	59.5
	0.20	3	0.5	9	2.3×10^6	820.			74.5
Alpha-dinitro-phenol	0.005	3	87.	3	9.6×10^8	2.7	2	7.4×10^7	4.1
	0.020	3	72.	3	1.1×10^8	8.1	5	2.5×10^8	5.5
	0.050	3	58.	10	1.7×10^8	33.5	5	1.5×10^8	5.4
	0.020	3	46.	10	9.5×10^7	51.5	4	8.4×10^7	8.5
	0.005	3	37.	10	2.3×10^9	25.1	5	3.1×10^8	12.5
	0.050	3	32.	10	6.6×10^7	56.0			8.5
	0.025	3	31.	9	1.7×10^9	14.2			12.5
	0.040	3	23.	10	4.8×10^7	25.0			8.5
	0.050	3	18.	10	9.1×10^7	37.4			5.5
	0.100	3	3.0	9	8.2×10^7	44.0	5	1.5×10^9	7.2
	0.100	3	2.6	10	7.3×10^7	97.0	5	1.4×10^9	7.3
	0.100	3	2.5	9	1.2×10^8	6.0			5.5
	0.100	3	2.4	7	3.5×10^7	65.5	4	8.4×10^8	7.6
	0.100	3	1.7	8	4.8×10^7	79.0	4	1.4×10^9	2.8
Trinitro-phenol	0.010	3	94.	8	1.9×10^9	14.9	5	1.3×10^8	14.3
	0.018	3	34.	8	4.9×10^8	97.3	6	1.1×10^8	9.3
	0.017	3	7.4	8	5.8×10^7	132.	6	5.8×10^7	3.4
	0.017	3	4.2	8	6.2×10^7	27.4	5	8.9×10^7	4.5
	0.018	3	1.9	8	1.9×10^7	68.4	6	7.8×10^7	7.7

TABLE 1 (*continued*)

Chemical	Concentration %	Treatment (hours)	Survival %	Treated			Control		
				No. of plates	Total no. of bacteria	Mutants per 10^8 bacteria	No. of plates	Total no. of bacteria	Mutants per 10^8 bacteria
Sodium salicylate	2.0	3	62.	5	4.2×10^7	16.7	5	7.0×10^7	20.8
	1.0	3	38.	3	9.5×10^7	5.3	5	4.0×10^8	5.8
	2.0	3	10.	8	3.2×10^8	0.0	5	2.0×10^9	0.7
	3.0	3	0.03	10	2.2×10^6	0.0	5	4.0×10^8	5.8
Carbamate (methyl)	4.0	48	100.	7	3.0×10^8	8.0	5	1.7×10^7	6.1
	8.0	3	69.	8	1.4×10^9	14.4	6	7.8×10^7	7.7
	7.0	25	10.	8	1.8×10^8	5.5	6	1.3×10^8	10.6
	8.0	24	5.5	8	1.1×10^8	9.1	6	8.6×10^7	19.9
	8.0	24	4.6	8	8.6×10^7	4.7	6	7.0×10^7	5.7
	7.0	29	2.2	5	2.4×10^7	12.6			10.6
Carbamate (ethyl) =Urethane	5.0	3	62.	10	1.4×10^9	18.6	5	1.1×10^8	7.9
	6.0	3	56.	8	1.3×10^9	26.5	5	1.4×10^8	16.8
	4.0	3	27.	10	4.8×10^8	8.4	6	1.8×10^8	5.5
	3.5	3	25.	10	4.5×10^8	6.6			5.5
	7.0	3	10.	8	1.7×10^8	41.4	5	1.1×10^8	12.1
	7.0	3	8.3	8	1.7×10^8	53.3	5	1.3×10^8	14.3
	7.0	4	8.2	8	2.3×10^8	22.5	5	1.7×10^8	9.2
	7.0	4	4.7	7	5.6×10^7	98.5	5	8.5×10^7	8.3
	7.0	4½	2.7	8	2.0×10^7	40.0	6	5.8×10^7	3.4
Carbamate (*n*-propyl)	2.15	3	52.	7	4.6×10^8	28.0	6	7.6×10^7	6.6
	2.00	3	50.	8	7.0×10^8	11.9	6	1.1×10^8	7.5
	2.00	3	35.	8	3.6×10^8	29.9	6	7.8×10^7	6.4
	2.00	3½	32.	8	6.6×10^8	8.3	6	1.6×10^8	3.9
	2.00	3½	30.	8	7.2×10^8	10.6	6	1.8×10^8	2.2
	2.10	3½	29.	6	5.2×10^8	16.4			2.2
	2.10	3	24.	8	2.7×10^8	23.0	6	8.5×10^7	9.4
	2.10	3½	4.1	8	9.8×10^7	17.4	5	1.5×10^8	3.3

TABLE 1 (*continued*)

Chemical	Concentration %	Treatment (hours)	Survival %	Treated			Control		
				No. of plates	Total no. of bacteria	Mutants per 10^8 bacteria	No. of plates	Total no. of bacteria	Mutants per 10^8 bacteria
Carbamate	2.10	3½	3.9	8	8.1×10^7	9.9			3.9
(*n*-propyl)	2.10	3	2.9	8	7.0×10^7	24.2			3.3
(*continued*)	2.10	3½	2.5	8	3.2×10^7	34.8	6	9.7×10^8	12.3
	2.10	4	1.4	8	3.4×10^7	23.8			3.3
Carbamate	0.50	3	85.	8	8.6×10^8	21.4	6	7.8×10^7	7.7
(*n*-butyl)	0.76	3½	44.	7	9.2×10^8	5.3	6	1.8×10^8	2.2
	0.75	3½	29.	8	7.0×10^8	5.6	5	1.5×10^8	3.3
	0.75	3½	20.	8	4.2×10^8	8.3	6	1.6×10^8	3.9
	0.75	3½	19.	7	1.7×10^8	20.9	6	7.6×10^7	6.6
	0.75	3½	17.	8	2.6×10^8	7.4	6	1.1×10^8	2.7
	0.75	3	16.	8	1.6×10^8	10.0	7	7.6×10^7	9.2
	0.80	3½	4.9	8	1.2×10^8	5.9			3.3
	0.75	3½	3.4	8	4.5×10^7	8.9	6	9.7×10^7	12.3
	0.75	3½	1.4	8	1.5×10^7	0.0	6	7.8×10^7	6.4
Carbamate	0.25	3	100.	8	2.0×10^9	8.4	6	1.6×10^8	3.9
(isoamyl)	0.30	3	100.	8	1.6×10^9	11.3	6	1.1×10^8	2.7
	0.35	3	57.	8	8.5×10^8	8.0			2.7
	0.35	3	54.	8	1.1×10^9	9.4			3.9
	0.36	3	26.	8	6.2×10^8	6.3	6	1.8×10^8	2.2
	0.38	3	6.3	8	1.5×10^8	7.2			2.2
	0.37	3	5.8	8	1.4×10^8	7.0			2.2
Neutral red	0.00025	3	50.	9	7.6×10^8	57.3	9	1.5×10^9	6.6
(in light)	0.00100	3	43.	9	6.6×10^8	118.			6.6
	0.00050	3	24.	8	3.2×10^8	122.			6.6
	0.00050	12	0.2	8	1.2×10^6	178.	8	6.7×10^8	10.8
Neutral red	0.00050	24	90.	9	4.2×10^8	17.2	6	4.6×10^8	10.1
(in the dark)	0.00050	12	86.	8	9.6×10^7	10.6	7	9.8×10^7	9.8

TABLE 1 (*continued*)

Chemical	Concentration %	Treatment (hours)	Survival %	Treated			Control		
				No. of plates	Total no. of bacteria	Mutants per 10^8 bacteria	No. of plates	Total no. of bacteria	Mutants per 10^8 bacteria
Neutral red (in the dark) (*continued*)	0.00050	24	83.	12	6.5×10^7	15.4	10	6.5×10^7	15.4
	0.00100	24	71.	10	4.6×10^7	17.4			15.4
	0.00050	12	70.	15	9.0×10^8	10.8	8		10.8
Acriflavine	0.0015	3	39.	10	4.8×10^8	78.3	4	1.0×10^8	14.4
	0.0030	3	28.	10	3.4×10^8	59.1			14.4
Caffeine alkaloid	2.10	3	72.	7	1.3×10^9	15.5	6	7.6×10^7	6.6
	0.60	48	44.	7	7.8×10^8	31.1	6	1.5×10^8	9.8
	2.10	24	34.	8	1.7×10^8	30.3	6	3.7×10^7	10.9
	0.90	48	15.	8	3.9×10^8	21.8	5	1.1×10^8	7.9
	1.00	48	15.	8	1.3×10^8	32.1	6	6.7×10^7	17.9
	0.50	48	12.	10	7.0×10^7	13.0	6	7.1×10^7	5.6
	0.60	48	5.1	8	9.3×10^7	34.6			7.9
	1.09	48	3.8	8	2.0×10^7	20.4	6	3.9×10^7	2.5
Necrosin	0.20	3	100.	8	4.6×10^8	23.6	6	1.4×10^7	6.9
	0.05	3	100.	8	4.2×10^8	26.0			6.9
	0.40	3	100.	8	1.2×10^9	16.4	6	1.0×10^8	9.9
	1.30	3	44.	8	8.5×10^8	17.8	6	1.4×10^8	8.4
	0.65	3½	3.4	7	3.9×10^7	172.	6	9.8×10^7	4.1

phenol (picric acid). The mutagenicity of phenol, however, was evident only after treatments that left very few survivors.

Sodium salicylate. Not mutagenic.

Carbamates. It was known from Oehlkers' work (1943) that some carbamates were able to induce chromosomal aberrations. Vogt (1948) had discovered that ethyl carbamate (urethane) showed mutagenic action in Drosophila. Since then, carbamates had been used by Bryson (unpublished) to induce mutations to phage resistance in *E. coli.* In our experiments, methyl carbamate gave negative results; ethyl and *n*-propyl carbamate gave good positive results; and *n*-butyl carbamate and isoamyl carbamate showed much weaker mutagenic effect.

Neutral red. Luther (1939) had found that neutral red was able to produce chromosomal aberrations. Buzzati (1947), treating Drosophila eggs with this substance, had observed an increase in frequency of mosaic flies. In our tests it seemed at first impossible to obtain low survival ranges, because treatment with concentrations higher than 0.001 per cent produced heavy clumping, and increases in length of treatment did not appreciably affect the number of survivors. Therefore no clear-cut positive results were obtained with the standard procedure. Since neutral red is a photodynamic substance, however, we then tried exposing the bacteria to light during treatment (a 60-watt bulb at a distance of about 10 cm), and finally got the desired survival ranges and with them a very large increase in frequency of mutants. Light had no effect on the controls. Similar results were reported by Kaplan (1948, 1949, 1950a, 1950b), treating *Bacterium prodigiosum* and *E. coli* with erythrosine.

Acriflavine. This substance was known to be a mutagenic agent for both bacteria and Drosophila (Witkin, 1947). Our tests also showed clear-cut positive results.

Caffeine. Caffeine had been found to be mutagenic for Ophiostoma (biochemical mutations) by Fries and Kihlman (1948) and for *E. coli* (mutations to phage resistance) by Witkin (Demerec, Wallace, and Witkin, 1948). Our experiments confirmed these results.

Necrosin. Necrosin is the name given by Menkin (1943) to the potent injurious euglobulin fraction of the exudate of inflamed tissues of vertebrates. A similar substance has subsequently been found by him in severely damaged tissues of some invertebrates (Menkin, 1949). The sample of necrosin used in our experiments was received from Dr. Menkin. It was found to be mutagenic, particularly in experiments where the proportion of survivors was low.

DISCUSSION

Streptomycin-dependent (Sd-4) bacteria, when plated on broth-agar medium without any streptomycin, undergo several divisions (residual growth) and then stop growing. The concentration of streptomycin in the medium in which the parent bacteria are grown is the most significant of the known factors influencing the number of divisions. For example, bacteria grown in broth medium containing 10 micrograms of streptomycin per milliliter, when plated on medium without streptomycin, will pass through 2-3 divisions; whereas bacteria grown in medium containing 25 micrograms of streptomycin will pass through 3-4 divisions. It has been found that very few, if any, nondependent back-mutants (background mutants) are present in cultures grown in broth with streptomycin, and therefore almost all such back-mutants appearing on the streptomycin-free plates can be assumed to originate during the residual growth. The almost complete absence of background mutants reduces the possibility that differential killing may be responsible for the increased proportion of back-mutants found among the survivors of treatment with certain chemicals; and this, together with the facts that residual growth can be dependably regulated in untreated material and that it allows expression of the back-mutations induced by treatment, makes the Sd-4 method especially suitable for studies of induced mutability.

Since spontaneous back-mutations occur during residual growth, any factor that increases this growth should increase the number of mutants. Therefore, in experiments such as these described here, it is necessary to be sure that any observed increase in mutant frequency is an effect of the treatment on the treated bacteria and not an effect of the treatment on the extent of residual growth. It is not likely that the chemicals used in these experiments would increase the residual growth. In the first place, they were usually applied in nonphysiological, toxic doses. Furthermore, the streptomycin requirement of the dependent bacteria is highly specific (Demerec, 1950), and ability to substitute for it would be quite unexpected in an unrelated chemical. However, even supposing that this had occurred, it would have been detected, either by the appearance of heavy residual growth on the plates of treated bacteria or through the microscopic check of the extent of residual growth that was made in all doubtful cases.

The analysis of data on the mutation-producing chemicals, presented in table 2, indicates that there were considerable differences of behavior among them. Some (ammonia, copper sulfate, and necrosin) were mutagenic only in treatments that left less than 5 per cent survivors; whereas others (hydrogen peroxide, formaldehyde, alpha-dinitrophenol, and neutral red in light) showed effects in treatments that allowed more than 50 per cent survival. Formaldehyde appears to be the most potent mutagen on the list. A treatment that left 61 per cent survivors resulted in 400 mutants per 10^8 treated bacteria; and two other treatments, each leaving 58 per cent survivors, gave 133 and 157 mutants, whereas in the controls there were only 25.2 and 15.5 mutants per 10^8.

Of the 31 chemicals listed in table 1, 19 were found to be mutagenic (table 2), and the following 12 were not: sulfuric, phosphoric, nitric, and hydrochloric acids; sodium and potassium hydroxides; mercuric chloride; silver nitrate; sodium silicate; sodium lactate; sodium salicylate; and methyl carbamate. Since the potency of the different mutagenic chemicals ranges from slight to strong, and, at least in the case of one mutagen that has been studied intensively (manganous chloride), can be shifted from one

TABLE 2

SUMMARY OF THE DATA ON MUTAGENIC CHEMICALS, SHOWING HIGHEST AND LOWEST FREQUENCIES OF MUTANTS OBSERVED IN EXPERIMENTS USING DIFFERENT TREATMENTS (EXPRESSED IN PERCENTAGES OF SURVIVORS)

	Survivors (per cent)			Controls
	50–100	5–50	less than 5	
	No. of mutants per 10^8 bacteria			
Boric acid	27	16–82	19–57	6–31
Ammonia	5	2–10	38–121	3–13
Hydrogen peroxide	20–29	121–124	27–175	5–7
Copper sulfate	6	4–21	40–56	2–21
Acetic acid	8	15–36	57	7–10
Lactic acid	...	11–40	11–35	5–27
Formic acid	18–20	24–44	38–39	3–15
Formaldehyde	133–400	120–326	560	12–79
Phenol	3–85	...	124–820	1–75
Alpha-dinitrophenol	3–34	14–56	6–97	3–13
Trinitrophenol	15	9–132	27–68	3–14
Carbamate, ethyl	19–27	7–53	40–99	6–20
n-propyl	12–28	8–30	10–35	2–12
n-butyl	21	5–21	6–9	2–12
isoamyl	8–11	6	7	2–4
Neutral red, light	57	118–122	178	7–11
dark	11–17	...	...	7–15
Acriflavine	...	59–78	...	14
Caffeine	16	13–35	20	3–18
Necrosin	16–26	18	172	4–10

extreme to the other by simple pretreatments of the bacteria, it seems very likely that the chemicals listed above as nonmutagens might become mutagenic if the treatment were made in a certain way. At any rate, examination of the list of mutagens in the table shows that mutagenicity does not belong to any one group of chemicals but is distributed widely among the different groups tested. Chemicals with such different properties as boric acid, ammonia, hydrogen peroxide, copper sulfate, acetic acid, formaldehyde, and phenol are on the list of mutagens. This fact, together with the indicated possibility that chemicals now considered noneffective would become mutagenic if appropriate conditions of treatment were found, leads us to conclude that genetic changes may be induced by most agents that are able to enter a living cell, upsetting some of its metabolic functions. It seems very likely that the genetic effect of a chemical is not direct but induced through changes in the cytoplasm, which in turn affect the genes. If this is so, it should be very difficult to establish what is the final reaction producing a change in a gene. It may well be that similar final reactions may be initiated by very different primary changes.

In this study we were considering back-mutations from Sd-4; and there is evidence to indicate that a majority of these may occur at one gene locus (Demerec, 1950; Newcombe and Nyholm, 1950). Several experiments by others, however, based on observation of mutations to Tl phage resistance, have tested the mutagenicity of a few of the chemicals we used. Witkin (Witkin, 1947; Demerec, Wallace, and Witkin, 1948) found that acriflavine and caffeine were mutagenic; and Bryson (in manuscript), in extensive experiments with several carbamate compounds, showed that ethyl carbamate (urethane) is mutagenic. It therefore appears very probable that the mutagenic effect observed by us was not specific, but that treatment with these chemicals increases the mutation rate of the whole genome.

It should be kept in mind that we are dealing here with genetic changes that occur spontaneously with a rate of the order of one per 10^8 cell generations, a considerably lower rate than could be detected directly in germ cells of multicellular organisms. Since each cell of a multicellular organism is a genetic entity, however, in which mutations may occur spontaneously or be induced by various agents, the likelihood of mutation in some of the somatic cells of a higher organism is good, if a large number of these cells are exposed to the action of a mutagenic chemical. This likelihood is further increased by the fact that each cell contains several thousand genes, in any one of which a mutation may be induced.

SUMMARY

Thirty-one chemicals, representing various organic and inorganic groups, were tested for ability to induce back-mutations from streptomycin dependence (Sd-4) to nondependence in *E. coli.* Nineteen were found to be mutagens. It was demonstrated that mutagenicity is not a specific property of any one group of chemicals, but appears among widely different groups. Chemicals having such different properties as boric acid, ammonia, hydrogen peroxide, copper sulfate, acetic acid, formaldehyde, and phenol were found to be mutagenic, indicating that genetic changes may be induced by many agents that are able to enter the living cell and upset its metabolic functions.

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AN INSTANCE OF AMIXIA BETWEEN TWO SPECIES OF LANDSNAILS (PULMONATA, HELMINTHOGLYPTIDAE)

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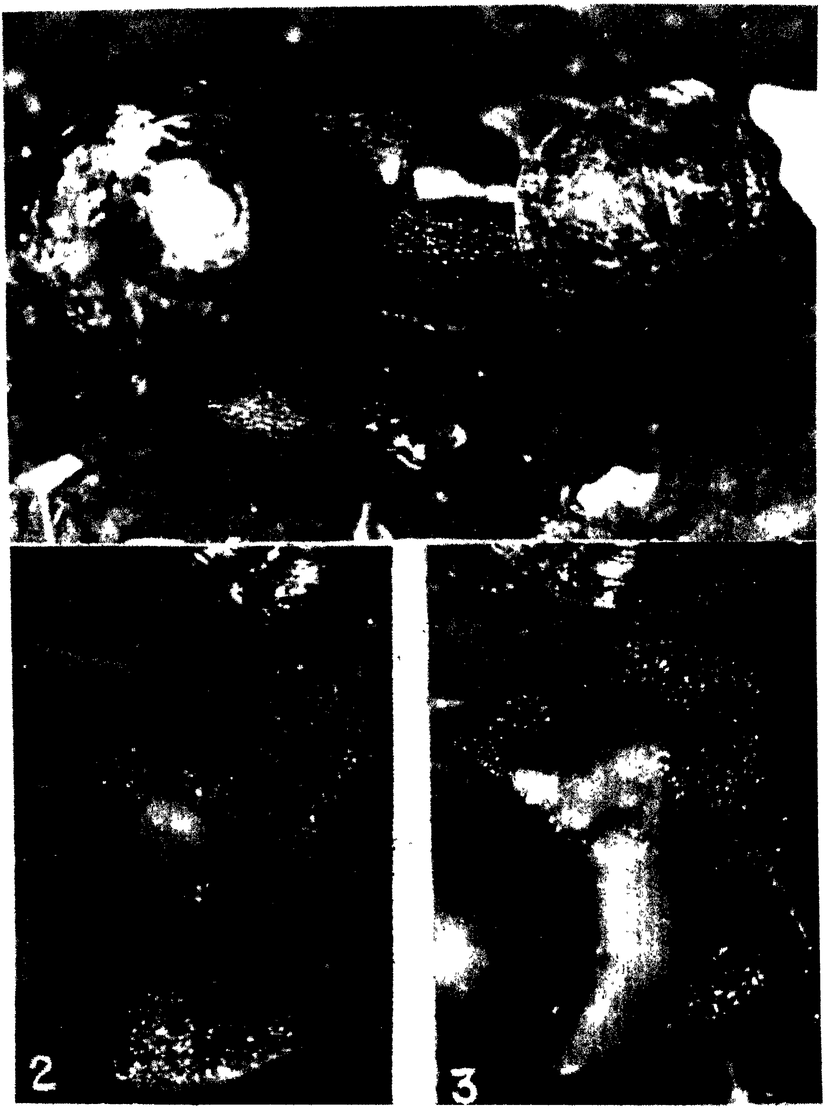
When one surveys the variants of the basic type of sex-organ exhibited by the numerous forms of Helminthoglypta, (Pilsbry, 1939) the functional properties of these organs as either panmictic or amictic forces of evolution present an interesting problem. The following data bear on the sexual isolation of two species, *Helminthoglypta californiensis vincta* (Valenciennes) and *H. dupetithouarsi* (Deshayes).

The specimens of *californiensis* were collected at the dumps north of Monterey, California, with E. P. Chace, who kindly showed me the special habitat preference of the species. The specimens of *dupetithouarsi* were secured from the pine-forested hills above Monterey. The lots of the two species were being kept together in a carton cage when the following observations were made.

A large *dupetithouarsi* was noted following at the tail-tip of a *californiensis* and, throughout the observations, was the more amorous of the pair. After the *dupetithouarsi* probed its dart into the side of the foot of the other species, the *californiensis* turned toward the aggressor and exserted the penis and atrium, as also did the *dupetithouarsi.* Copulation was not attempted, however, and the *californiensis* began to disengage from the courtship.

Since courting landsnails frequently wander apart but rejoin and finish mating, I have found it useful to reposition errants so that observations can be completed or mating-anatomies secured. Accordingly, the seemingly separating snails were artificially repositioned to determine if mating would continue and copulation eventuate. By gently picking the two snails up and placing them on a small, wet, glass plate, it was possible to gently slide the snails into the head-on position again whenever they had started separating. By such maneuvers the pair were kept in courtship, until it culminated in attempts at coitus. However the penis tip (verge) of the *dupetithouarsi,* although frequently protruded vigorously, failed to penetrate the orifice of the female organ of the *californiensis* and the latter's penis proved to be too short to reach the female orifice of the former.

An entire view of the attempted mating is shown in figure 1, the onset in figure 2, and the details of the attempted coitus in figure 3, in which the curved, finger-like dart-organ of the *dupetithouarsi* is in functioning position against the foot of the other snail, and the tapering upper lobe is the penis



FIGURES 1–3. The attempted coition between *Helminthoglypta dupetithouarsi* (Deshayes) and *H. californiensis vincta* (Valenciennes). The anatomical details are shown in figures 2–3 at greater enlargement (see text).

attempting ingress into the female organ of the *californiensis*. In figure 1 the snail to the left is the specimen of *Helminthoglypta dupetithouarsi* and the smaller snail to the right is the specimen of *Helminthoglypta californiensis*.

The observations ended when the *californiensis* retracted its sex-organs and turned away. It was seen to bear several deep (2–3 mm.) dart-wounds on the sole of the foot. Although generally present in recently copulated specimens of Helminthoglypta, the wounds in this individual seemed more conspicuous and were still evident the next day. On the fourth day after the attempted mating, the *californiensis* was found dead with its foot noticeably swollen. The primary cause of death seems to have been the dart-wounds, which may secondarily have become much infected. Having never previously noted dart-wounds to be mortiferous in Helminthoglypta subsequent to matings, it is possible that the mucus from the dart of the *dupetithouarsi* was harmful to the tissues of the other species, or that the nearly three-times-longer dart provided a path for infection into tissues not ordinarily penetrated or wounded.

I am much indebted to Dr. S. Charles Kendeigh of the University of Illinois for helpful suggestions in writing this paper. Further observations on the sexology of these species have been published elsewhere (Webb, 1942).

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LETTERS TO THE EDITORS

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A SUPPRESSOR MUTATION IN *ESCHERICHIA COLI**

Nutritionally deficient mutant strains of bacteria (auxotrophs) are often capable of undergoing "reversion," giving rise to strains having the wild type nutritional state (prototrophs). It is usually impossible to determine whether the prototrophs are true back-mutants, or whether the wild phenotype is restored by the action of an independent suppressor mutation.

An auxotroph requiring histidine was isolated from strain B/r of *Escherichia coli* (which grows well on minimal medium) after ultraviolet irradiation and layering.[1] Some time later, the histidineless strain was irradiated with ultraviolet and subjected to penicillin screening.[2,3] Among the mutants isolated was one requiring serine or glycine in addition to histidine. This diauxotroph, strain M2, was thus characterized by two growth factor requirements acquired separately and serially, presumably by two mutational steps.

A study of the reversion behavior of strain M2 revealed an interesting departure from the usual result with diauxotrophs. Prototrophs were obtained with a frequency of about $1/10^7$ bacteria plated in minimal medium. When the strain was plated in minimal medium supplemented with histidine, or with serine, prototrophs were obtained in addition to the expected single reversions, and the rate of double reversion was found to be about equal to the rate of single reversion from either of the separate requirements. The prototrophs obtained, therefore, could not possibly result from coincidental back-mutation of the two independent requirements. The quantitative results strongly suggested a suppressor, as did the fact that the growth rate of the prototrophs obtained from M2 was considerably slower than that of the wild type parent strain in minimal medium.

The suppressor hypothesis was tested by attempting to induce reversion of the postulated suppressor, thereby releasing the double requirement for histidine and serine or glycine in a single step. A prototroph strain, M2P, obtained from a plating of M2 on minimal medium, was irradiated with ultraviolet light and put through the penicillin procedure. Mutants were screened on minimal plates supplemented with both serine and histidine, so that any variant requiring one or both of these growth factors could be detected. Colonies appearing on these plates were analyzed, and eight out of 130 tested proved to require *both* histidine and serine or glycine. As a control, the wild type strain was irradiated and penicillin-screened in exactly the same way. Three hundred twenty colonies appearing on the serine-histidine supplemented plates were analyzed, and none was found to require both growth factors. These results can best be interpreted by assuming that the prototrophs arising in strain M2 owe their wild phenotype to the action of an ultraviolet-reversible suppressor, capable of overcoming the two independent metabolic blocks. The biochemical basis of this system is obscure, but a possible explanation could be the following: (1) the mutation to histidine requirement blocks histidine synthesis by the production of an inhibitor; (2) the mutation to serine or glycine requirement blocks serine-glycine synthesis by an increase in the concentration of the same inhibitor; (3) the suppressor mutation eliminates or otherwise neutralizes the inhibitor.

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GENE MECHANISMS AND BEHAVIOR

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The demonstration of intraspecific differences in behavior which are due to genetic factors has been demonstrated by several workers. A bibliography of such studies up to 1946 has been given by Hall (1947). In animals such as the rat and dog, variability has been clearly shown in the motivational and temperamental aspects of behavior, conditioned reflex formation, and in the type of social relationships established (Scott, 1950; Searle, 1949; Stockard, 1941; and Thorne, 1944). It is possible that there are also differences in perceptual ability, in rate of learning, and in the ability to analyze complex problems; but difficulties in excluding the effects of emotional upsets in test situations make it hard to obtain conclusive proof. These results of animal studies are in some disagreement with the results of studies on human identical twins. A recent text on human genetics (Stern, 1949) summarizes many researches with the statement that the balance of evidence favors a greater genetic effect on "intelligence" as measured by standard I. Q. tests than on "temperament" as determined by objective rating scales. The contradiction may be more apparent than real, and further studies may reconcile the discrepancies. It should not be necessary to belabor the point that we are dealing with a field in which rigorously controlled experimentation is difficult. Behavior traits are highly modifiable by the environment, and environmental effects may be very subtle and difficult to detect. There is, however, no need to be excessively pessimistic. Experiments such as those of Scott (1950) are extending our knowledge of the range of phenotypic variation which a particular genotype will permit. Sociobiological and psychobiological studies of this kind may well have important consequences in education and social thinking.

To complement behavior studies, however, there is place for research on the processes which lie between the transmission of genes from parent to offspring and the appearance of a characteristic behavior pattern. It is a far cry from minute particles of nucleo-protein in an egg or sperm to behavior described in terms of responses of aggression, timidity, courtship, or the ability to solve differential equations. To some it may seem presumptuous to attempt to bridge this gap at the present time. We are a long way from understanding how genes control relatively simple metabolic processes, while attempts to find the organic basis of psychological phenomena have fallen short of the rosy hopes of a host of investigators. Nevertheless, I believe that there is value in a brief survey of our present state of knowledge which may point the way to profitable avenues of investigation.

Studies of gene control of the chemical characteristics of microorganisms, plants, and animals (Beadle, 1946; Catchside, 1950; Lincoln and Porter, 1950; Wright, 1941), and on gene mechanisms and morphogenesis of higher forms (Dunn, 1949; Gluecksohn-Schoenheimer, 1945; Grüneberg, 1947) are also pertinent to the relationship of genes to behavior. Physiological psychology is active with ideas concerning the energetics of human behavior (Freeman, 1948); the description of brain function in terms of the unities found through factor analysis (Halstead, 1947); theories of feedback and servo-mechanisms which have led to a new scientific field called cybernetics (McCullough, 1950; Wiener, 1948), and attempts to interpret the laws of behavior in terms of neurophysiological principles (Hebb, 1949; Lashley, 1949). Paralleling such theoretical approaches are many experimental studies on brain-operated animals covering emotions (Bard and Mountcastle, 1948; Speakman and Babkin, 1950), delayed response (Pribram, 1950), learning (Lashley, 1944) and a great deal of interest in problems of nerve metabolism. The effects of hormones on behavior have been admirably summarized recently (Beach, 1948; Collias, 1950), so that this possible means of genetic control of behavior can be objectively evaluated. All of these developments have an influence on our way of thinking about gene mechanisms and behavior.

Although behavior is a characteristic of the organism as a whole, and cannot be considered as a property of any one system, it is certain that the nervous system is central to the integration of responses into coherent patterns. Hence, it is profitable to emphasize the relationship of genes to the nervous system.

Expressed formally, gene action may affect behavior through direct or indirect action on the central nervous system. Direct effects include: control of the number of neural elements; determination of the interconnections of the neural elements; and modifications of the chemical and physical characteristics of neurons, so that they are more or less readily modified in the course of their functioning. In addition, there are secondary effects. Genes may determine the ability of an organism to utilize available nutrients, and, therefore, the energy available to the control system. Another type of control involves the effect of genes on the formation of chemical agents such as hormones, which affect the excitability of specific pathways in the central nervous system. Genes may also control the characteristics of exteroceptors (as in red-green color blindness), and thus influence the amount of information which the nervous system receives from outside the organism. Finally, they may influence the effectors, so that behavior patterns requiring specific movements are facilitated or made impossible, and feed-back to the central nervous system is thereby modified. The direct effects of genetic sensory and motor defects on behavior are often obvious, and appear to be similar to those of the same defects of non-genetic origin. The indirect effects are primarily related to the fact that defective animals are at a great disadvantage when they are forced to compete. When protected from competition such defective individuals may adjust well emotionally and show no behavior deficits which are not clearly related to their primary disability. In the remainder of this talk we shall be primarily concerned with evidence for effects upon the functions of the central nervous system.

A complete case must include a demonstration that a particular physical character is gene controlled, and that a particular behavior pattern is a unit which is uniquely determined by the physical character. No such case is now known except for pathological conditions.

Turning to the direct effects of genes on the nervous system, one would expect that an increase in the number of neural elements would increase the flexibility of the control system and allow it to respond more selectively to variations in input signals. In general the brain-weight/body-weight ratio of vertebrates is positively correlated with psychological complexity (Lashley, 1947). No marked relationship between brain mass and behavior has been found. within a species, though the work of Spitzka and Basset as quoted by Lashley indicated, in men and rats respectively, a tendency for superior performance to accompany increased brain weight/body weight ratio.

There are many cases of neurological defects in genetically controlled strains of animals. Examples include ataxic and epileptic rabbits, ataxic pigeons, waltzing, shaker and other choreic mice. (For details see Riddle, 1917; Nachtsheim, 1939; Sawin, Anders and Johnson, 1942; Grüneberg, 1943). In some cases (e.g. rabbit ataxia, Anders, 1945) these behavior mutants suffer from deficiencies in specific tracts of the nervous system. In the waltzing mouse no specific defect has been yet demonstrated as an essential component, although most waltzers have pathological conditions in the region of the acoustic nerve. In still other cases (rabbit epilepsy) the disorder is classed as functional because no structural pathology is known. These mutants may also differ in their general level of activity. For example, the waltzing mouse is extremely active and the males are recorded as very aggressive. Waltzing and shaker mice have good vitality and fertility, but other defects, such as rabbit ataxia, are semi-lethal and result in progressive deterioration and death before reaching the age of reproduction. It is unwise to draw too strict a line between the disorders characterized by known pathology, and those which are called functional, since the latter undoubtedly involve derangement of structure at a finer level, colloidal or molecular.

There are few quantitative studies on the nervous system of different genetic strains of animals. Smith (1928) has described variations in the number of mitral cells in the olfactory bulbs of two strains of the albino rat, but he makes no reference to any behavioral differences which might arise from this condition. In general, it may be stated that careful quantitative studies almost always show great individual variability in the nervous system. This is well shown by a quotation from Lashley and Clark (1946) regarding their work on the structure of the cerebral cortex of the spider monkey: "There is scarcely a quantitative histological character which does not vary by 25 to 100% in local regions." These observations were made on presumably normal specimens, but no genetic studies were undertaken; nor is the behavioral significance of such quantitative morphological differences clearly established. A certain amount of structural variability occurs in strains of mice inbred for upwards of 100 generations. Characters such as the distribution of pigment rings in the tail and ventral spotting retain marked variability in essentially homozygous strains (Murray and Green, 1933). Perhaps the differences in cell arrangement of the cerebral cortex are produced by random mechanical factors rather than by gene differences. The research necessary to show the origin of this variability should definitely be undertaken.

In summary, therefore, the existence of individual differences in the structure of the central nervous system is well established, but their importance for differences of temperament, social behavior and intelligence is unknown, and genetic control has been shown only for gross neurological defects.

Genetic control may also operate through the production of variations in the autonomic nervous system. Lacey (1950) has demonstrated that individuals have characteristic modes of physiological response to stressful situations. One person may react chiefly by a rise in systolic blood pressure, another by changes in heart rhythm, and still another by changes in skin resistance. The important role of the autonomic system in learning simple habits has been shown by Solomon and Wynne (1950) who found that sympathectomy in dogs retarded the formation of an avoiding response. It is likely that feed-back from autonomic effectors is one of the factors influencing the modifiability of the central nervous system. Various workers in the field of individual physiology have postulated that genetic factors produce the individuality (Wenger, 1948; Jost and Sontag, 1944). Recent studies on pure breeds of dogs have established the fact that each breed has a characteristic physiological profile, which appears to represent differences in autonomic functioning (Fuller, in press). All of this evidence suggests that genetically controlled autonomic patterning may affect learned behavior, but intensive individual studies, in which all variables are controlled, are needed to confirm the hypothesis.

The question of genetic effects on the characteristics of the individual neurons leads to a consideration of individual biochemistry and metabolism (Keys, 1949; Williams, 1946). Recently the hypothesis has been put forward that alcoholism may be produced by a hereditary inability to utilize specific nutrients, and the term "genetotrophic disease" has been introduced to describe such cases (Williams, Berry and Beerstecher, 1949). For many years we have known of phenyl-pyruvic amentia, a type of hereditary feeble-mindedness attributable to the failure of a specific enzyme to be formed in the brain. Here, again, we deal with a gross disorder sharply separated from the normal. Utilizing another gross deficiency, Koch and Riddle (1919) found that the brains of ataxic pigeons showed marked chemical deviation from normal, and the brain of the ataxic rabbit has been shown to be deficient in free amino acids (Roberts, Frankel and Harman, 1950). In chemical studies, as in anatomical ones, it is relatively easy to find evidences of genetic factors operating to produce gross pathologies but the significance of variability in the normal range has not been shown.

A promising tool for investigating the physiological genetics of behavior is the sound-induced convulsion characteristic of certain strains of mice when they are stimulated by a loud, high-pitched sound. Susceptibility to these seizures is definitely a genetic trait (Hall, 1947; Witt and Hall, 1949; Fuller, Easler and Smith, 1950), and is not merely a function of some extrinsic factor such as middle ear infection (Miller and Zamis, 1949). Animals of high and low susceptibility strains appear essentially normal in ordinary laboratory situations, although the highly susceptible strain is considered slightly less vigorous. The DBA strain, a highly susceptible strain, is also high in mammary tumor incidence, but other high mammary tumor strains have been found to be resistant to seizure. Young DBA mice, when exposed to sounds above 5000 cycles frequency at intensities near 100 decibels, usually go into a fit of wild running which terminates in a convulsion, often fatal. Ginsburg, surveying the literature on rat seizures, noted that many of the substances whose deficiencies increased susceptibility functioned in the body as enzymes. In a series of researches Ginsburg (1949) and his co-workers (Ginsburg, Ross, Zamis and Perkins, 1951) have investigated the physiological and chemical correlates of susceptibility. As a result of these studies Ginsburg has postulated that a contributing factor to seizure susceptibility is a deficiency in carbohydrate metabolism. This may be genic in the case of the DBA mouse, or nutritional in the case of the rat which is deprived of thiamine or magnesium. Deficiencies in enzyme systems due to genes are well known in *Neurospora* and relative deficiencies in the enzyme of a mammalian brain may be the cause of increased susceptibility to sound-induced seizures. Direct proof of this hypothesis still requires demonstration of metabolic differences in the nervous systems (or at least in certain parts of the nervous system) between susceptible and non-susceptible strains.

Closely allied to the gene-enzyme problem is the more general problem of the role of genes in protein synthesis. A physico-chemical theory of learning which has recently been developed by Katz and Halstead (1950), attempts formally to relate gene action and the type of change in the nervous system which accompanies learning. Their model of learning is based upon protein molecule denaturation by electrical fields set up during transmission of nerve impulses. The extreme diversity of the protein molecule and the well-known specificity of molecules formed by different genetic systems, for example the Rh blood groups and the blood groups of cattle (Irwin, 1947; Stormont, 1950), give this theory a definite plausibility.

Neurons formed by the action of one set of genes might be composed of proteins which were more readily denatured than proteins formed by another set of genes. Psychologically this would result in more rapid learning. Furthermore, these denatured molecules might be highly stable, and the learned behavior would persist; or unstable, and it would rapidly become extinct. A hypothesis of this type involves imagination which goes beyond proven facts, but Katz and Halstead have suggested experimental means of testing the theory, and we may hope that it will stimulate fruitful research.

Turning to indirect effects of genes on the nervous system, the role of the endocrine glands must be considered. Beach's (1948) monograph enumerates many researches concerned with endocrine effects. If we take as an example the action of the sex hormones, it is obvious that what is essentially the same chemical substance produces quite different courtship behavior in various species of amphibia, birds and mammals. Conversely, even within the same individual, typical male and female behavior may be produced by appropriate treatment with androgens or estrogens. The control of sex behavior by hormones is more complicated than a simple channelling into male patterns by male hormones, and into female patterns by female hormones, but numerous experiments have shown that genetic potentialities which would normally never be expressed may be elicited by hormone administration. In experimental reversal of sex behavior, genetic specificity is not lost. The androgen-treated bitch behaves like a male dog, and not like some other species. The importance of the somatic substrate is further shown in experiments by Grunt and Young (1950) who castrated male guinea pigs of high and low sexual activity. Both groups fell to a very low level of sexual activity. When the subjects were placed on hormonal therapy with identical dosage, the amount of sex behavior increased in both groups, but high-drive animals returned to a high level and low-drive animals recovered only to their previous low level. A somatic factor of target organ sensitivity appears to be the most likely explanation for this finding.

Space will not permit an exhaustive consideration of genetics, endocrines, and behavior. Certainly, quantitative differences in hormone administration (Beach and Holz-Tucker, 1949) will have quantitative effects on behavior. The sexual activity of male rats increases with higher levels of testosterone injections. However, variation in tissue responsiveness to hormones appears to be widespread. The schizophrenic (Hoagland, 1950) has adrenals which are markedly unresponsive to ACTH, and a genetic factor has long been suspected in this disease. Speirs (personal communication) working at the Jackson Laboratory has found great variability in the eosinopenic response of various strains of mice when injected with corticoids. The mouse strains are also known to differ in their storage of adrenal lipids (Vicari, 1943). Although androgens are known to be important in producing so-called spontaneous fighting in mice (Beeman, 1947), C3H and C57 black mice, which differ in fighting ability (Ginsburg and Allee, 1942) have similar levels of 17-keto steroid excretion (Karnofsky, Nathanson and Aub, 1944). Genes may affect behavior through control of the amount of hormones secreted, but probably a more important type of control is through modification of neurones and other target organs making them more or less sensitive to hormones, and thus altering the threshold of excitation of certain key circuits in the nervous system.

Another hypothesis for the mechanism of gene action on behavior is that genes affect the rates of physiological reactions in the nervous system. This theory is supported by evidence that brain wave frequencies have a hereditary basis (Lennox, Gibbs and Gibbs, 1945). A similar concept is familiar to students of growth, since morphological variability has been attributed to genetic control of rates of growth in specific centers (Huxley, 1932; Sawin, 1945). Excellent material for the study of gene-controlled time constants in the nervous system is found in the various mouse strains susceptible to sound-induced convulsions. It has been found that the latency of these seizures, that is, the length of auditory stimulation necessary to produce a convulsion, is shorter in strains with high susceptibility (Fuller, Easler and Smith, 1950). This led to the hypothesis that auditory stimulation induces at least two processes; one, excitatory, culminating in a violent spasm or convulsion; the other a process of accommodation which raises the threshold to convulsions. We do not have any definite evidence as to the nature of these processes. They may be primarily chemical, involving the production and hydrolysis of a substance like acetyl-choline (Freedman, Bales, Willis and Himwich, 1949); or physical, involving changes in the electrical properties of neurons which produce changes in the excitability of motor centers; or physiological, involving reverberating circuits with long persistence. The final result of prolonged loud auditory stimulation depends upon the outcome of a race between two opposing processes. If the excitatory process overtakes the accommodation process a convulsion occurs; if accommodation stays ahead, there is no convulsion, although preconvulsive behavior may be observed (figure 1). The line between convulsions and non-convulsions is sharply drawn, so that the outcome of the race can be accurately determined.

Fuller, Easler and Smith applied Wright's (1934) concept of developmental thresholds to their data on audiogenic seizure and concluded that "what appears to be a character of alternative expression is in reality a quantitative character which varies over a wide range." The general theory of heritability of threshold characters has recently been discussed by Dempster and Lerner (1950). These authors use the term *p scale* for the determination of a character in terms of its probability of appearance; for example the risk of a convulsion when a mouse is exposed to a standard auditory stimulus. The term *x scale* is used for the continuously variable factor which presumably underlies the character which can be directly measured on the *p scale.* The evidence is very strong that one underlying variable determining susceptibility to audiogenic seizures in mice is a rate

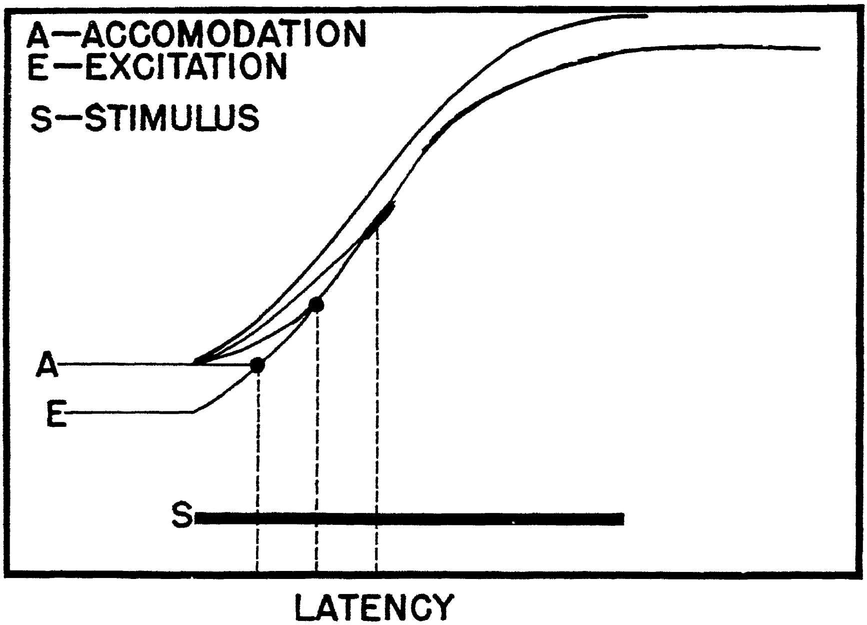

FIGURE 1. The relationship of stimulus, accommodation rate, and latency of convulsion in audiogenic seizures of mice. At the onset of stimulation, the level of excitation rises rapidly to a maximum. At the same time a process of accommodation starts at a rate depending upon the genotype of the mouse. The figure shows four different rates of accommodation; the slowest results in a very small latency; the fastest prevents the occurrence of a convulsion.

of accommodation which is inversely related to measurements of latency. Convulsion latency is remarkably constant under various experimental conditions in both rats and mice. Morgan (1941) and Wiener and Morgan (1945) found that latency was unaffected by intensity and quality of stimulation, or by cortical lesions, although these factors influenced the incidence of convulsions. Convulsions are always preceded by a period of wild running lasting from two or three seconds up to as much as thirty seconds. Following a suggestion of Dr. Ginsburg, strain *A* mice (34 days old at the beginning of the experiment) were exposed to auditory stimulation in the usual manner, but were forced to run by pushing them with a small paddle whenever they attempted to stay in one place. The animals were divided into two groups. Group I was scheduled: Agitation trial, 48 hours, non-agitation trial, 48 hours, agitation trial, until 8 trials were given. Group II was scheduled the same, except that the odd numbered trials were "*non-agitated,*" and the even trials were "*agitated.*" No significant differences were found between the groups. Each animal served as its own control, and the data were analyzed for relationship of agitation to the seizure risk, to seizure latency and to the occurrence of the first convulsion. There was an indication that under the conditions of the experiment, susceptibility increased following the first convulsion. The results were:

	Trials	Convulsions	First convulsion	Mean latency
Agitation trials	92	55	19	62.1 ± 2.8
Non-agitation trials	92	38	7	63.3 ± 4.0

There is no difference in latency, although agitation significantly increases both convulsion risk of a given individual ($p < .01$) and the likelihood of a convulsion in a previously resistant animal ($p < .02$).

All this evidence indicates that the mechanism determining latency is near the end of the chain of events which culminates in a convulsion, and that it is relatively insensitive to experimental manipulation. To date, the genetic effect is the only variable known to modify convulsion latency in mice, though it is likely that experimental control could be produced by utilizing the same physiological means which are employed by the genes. Some function of latency seems to be the best candidate for the *x scale* variable.

Recent experiments (table 1) involving crosses between three strains of mice, DBA/2 with high susceptibility, A with moderate susceptibility, and C57BL with extremely low susceptibility confirm the threshold hypothesis which was originally based only upon crosses between the first and third strains. The adjusted mean latencies recorded in this table are based upon a probit transformation of the data, under the assumption that non-convulsers have a presumptive latency which cannot be directly measured, but can be calculated if a normal distribution of latencies is assumed. Both measures of latency are negatively correlated with convulsion risk, but bear no relationship to the incidence of fatal convulsions. A separate genetic and physiological system appears to be involved in protection against the stress of undergoing a convulsion. Although the hypothesis that genes control convulsive behavior and the outcome of convulsive behavior by affecting the relative rates of accommodation is strongly supported by these findings, this type of experiment does not isolate the specific processes which are involved. Direct physiological and biochemical observations will be necessary.

TABLE 1

AUDIOGENIC SEIZURES IN VARIOUS INBRED STRAINS AND THEIR HYBRIDS.

Cross	Convulsion risk	Mean latency seconds	S. D. of latency	Adjusted latency	Deaths per convulsion
DBA × DBA	.92	35.8	12.5	38.3	.87
DBA × A	.91	35.6	16.6	39.4	.21
DBA × C57	.76	52.1	9.7	56.5	.59
A × A	.11	61.8	12.2	75.7	.00
A × C57	.045	54.6	...*	est. 74.6	.57
C57 × C57			No convulsions		

*Too few cases to compute a standard deviation.

To summarize briefly, genes might act on behavior mechanisms at the level of organs, cellular arrangement or molecular structure. Gross organ defects influence behavior, but many behavioral differences are uncorrelated with easily observed structural differences. There is a great deal of evidence for individual differences in the cellular arrangement of supposedly normal nervous systems, but the genetic control of these variations and their significance for behavior have yet to be worked out. Variations in autonomic patterns have been shown to have a genetic basis and are important in behavior, but correlations within an individual organism have not been made.

At the molecular level there is a great deal of presumptive evidence that the sensitivity to specific hormones and other physiologically active substances varies between individuals and between genetic strains of mammals. Students of behavior should make wider use of these cases of strain differences turned up by biologists. In the case of sound-induced seizures of mice, there is evidence for some sort of neural deficiency in susceptible mice. This deficiency is not absolute but alters the time constants of excitatory and adaptive responses so that a stress situation produces an explosive breakdown in certain genetic types. These genetically determined time constants are much longer than those of single neurons, which are usually measured in milliseconds, or of the "brain waves" of the cerebral cortex, which are measured in cycles per second. Their very slowness makes them particularly convenient for chemical and physiological analysis.

It is still a long way from nucleoprotein molecules to the behavior patterns of mating or of problem solving. The gap can be closed only by detailed exploration of promising experimental materials—animals which are well controlled genetically and well understood developmentally, physiologically and psychologically.

These problems are difficult, but they have a peculiar fascination. Ten years from now it should be possible to give a much more definitive account of gene mechanisms and behavior—one which will be more instructive to the reader and more satisfying to the writer.

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INTERSPECIFIC COMPETITION BETWEEN DROSOPHILA FUNEBRIS AND DROSOPHILA MELANOGASTER

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In the "Origin of Species," Darwin developed the concept of natural selection as the mechanism of speciation. As one phase of natural selection, the competition of organisms with one another was cited. This competition is primarily the result of the high reproductive potential of all species compared to the environmental capacity to support them. The biotic environment determines the extent of the competition, which is therefore governed by the numbers of organisms present. Since the requirements of members of the same species are most similar, Darwin suggested that intraspecific competition would be most severe, with the competition lessening, the greater the differences between various groups in their demands upon the environment.

The studies of competition since this first rather general statement of the problem have led to certain theoretical and experimental conclusions. On the basis of theoretical equations, Lotka (1925) and Volterra (1926), (see also Ross, 1908), independently predicted that two types with identical needs and habits cannot survive in the same place if they compete for limited resources. If both types are found together in nature, they must differ in their ecology, or else there are fluctuations in the environment, favoring first one and then the other competitor. Intraspecific competition between mutants of the same species has repeatedly shown that the wild type genes will survive the mutant alleles in populations of *D. melanogaster* (Lutz, 1911; L'Heritier and Teissier, 1937; Reed and Reed, 1950). An unforeseen exception to this rule is to be found in the survival of two types because of the superiority of the heterozygotes over the corresponding homozygotes for inversions in *D. pseudoobscura* (Dobzhansky, 1947). The survival of more than one type maintains genetic variability in the population.

Experiments with interspecific competition between similar species have also demonstrated the validity of the theory (Gause, 1934; Crombie, 1947; Zimmering, 1948). Careful analysis of the instances in which both species survive has shown that they occupy slightly different niches (Gause, 1934; Crombie, 1947).

The importance of these results to evolutionary theory is considerable. Organic evolution has been marked by the repeated divergence of new lines ("adaptive radiation"), which have invaded new habitats, and also by the evolution of more "efficient" types, which have replaced existing forms.

The extent to which competition has been responsible for these trends can not be readily estimated, but the conclusion that it has been a factor seems inescapable.

METHODS AND RESULTS

Since the pioneer work of Pearl (1926), Drosophila populations have frequently been used to study the dynamics of populations (L'Heritier and Teissier, 1937; Bodenheimer, 1938; Dobzhansky, 1947; Sang, 1950; and many others). In many early experiments, the flies were placed in a closed system, and the course of events was followed to the exhaustion of the food supply and the death of the flies. More recently, maintenance of the populations has been made possible by the use of population cages (Dobzhansky, 1947) or population bottles (Reed and Reed, 1948) to which food can be added at intervals.

L'Heritier and Teissier (1935) observed that mixed populations of *D. melanogaster* and *D. funebris* in population cages reached an equilibrium with a large population of *D. melanogaster* and a small population of *D. funebris*, no matter what their initial proportions. They noted the inconsistency of their results with the mathematical theories of competition and with the results of Gause. The observation that stocks of *D. funebris* survived longer in a bottle than a pure culture of *D. melanogaster* raised the question of whether this predominance of *D. melanogaster* in mixed populations would hold under all conditions.

Therefore, five mixed populations were started in population bottles with five males and five females of each species. The Oregon R strain of *D. melanogaster* and a local strain of *D. funebris* were used. The number of populations under study can be readily doubled at any time by attaching a bottle of fresh food to each bottle of the population unit. This procedure was occasionally followed to make additional populations available for study. The food consisted of the standard cornmeal medium (Demerec and Kaufmann, 1945) seeded with brewer's yeast. The temperature throughout was 20 ± 0.5°C.

The course of events can be followed in table 1 and figure 1. The data consist of samples of the mixed populations taken to determine the relative proportions of each species, and are not the total number of adult flies in the populations. Both species have coexisted in the population bottles for almost two years. The "equilibrium," however, is clearly not stable, but may undergo wide variations. These fluctuations can be correlated quite clearly with the age of the food. When fresh food was added, the proportion of *D. melanogaster* increased; as the food became older, the proportion of *D. melanogaster* declined. No food was added to two of the units, and the final populations were composed only of *D. funebris* before they died out. This result was confirmed with eight more mixed populations, a total of ten altogether. Addition of food before the extinction of *D. melanogaster* resulted in an increase of this species. If the food was added at regular intervals, and the counts made at about the same relative time in the cycle of food addition, a fairly stable equilibrium appeared to exist with *D.*

TABLE 1

COMPETITION IN POPULATION BOTTLES BETWEEN *D. MELANOGASTER* AND *D. FUNEBRIS*

Day	Populations	Sample n	Percent *D. funebris*
0	5	...	50.0
14	5	771	5.3
21	5	1832	2.0
28	5	1470	1.9
37	5	566	33.0
54	4	195	16.4
61	4	123	93.5
72	2	138	94.2
84	2	262	27.5
109	2	40	67.5
174	4	491	4.0
207	4	341	6.5
247	4	444	6.3
271	6	455	3.5
315	6	670	7.2
347	6	763	3.1
387	6	472	11.0
452	6	116	12.1
567	3	202	8.9
649	4	100	30.0

melanogaster predominating. This situation has persisted for more than a year. However, during this period, two units became pure *D. funebris*, and another pure *D. melanogaster*, indicating the precarious nature of the equilibrium. The losses probably occurred more or less by chance when the population of the lost species had been reduced by unfavorable conditions.

The data show that an equilibrium does, in fact, exist between two species of the same genus living in a system with a volume slightly greater

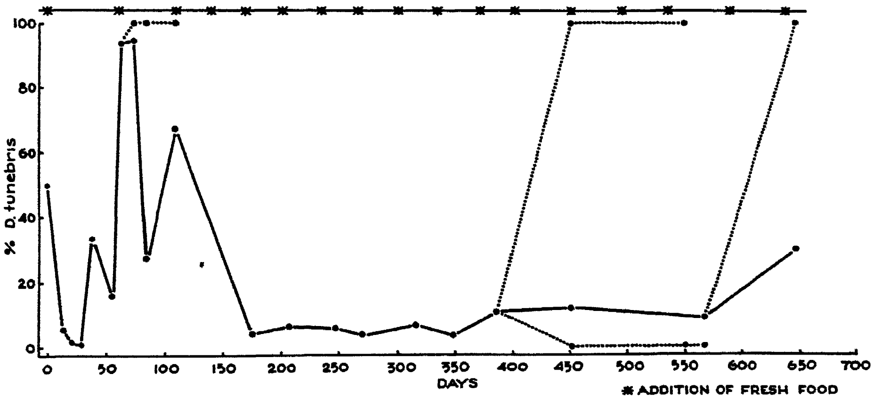

FIGURE 1. Competition between *D. melanogaster* and *D. funebris* in population bottles. No food was added to the two units reaching 100 per cent *D. funebris* at 72 days.

than a pint and drawing on the same basic source of energy. The next question to be considered is whether this result is an exception to the mathematical conclusions of Volterra and Lotka that species in direct competition with one another can not coexist.

It seems clear that even within this small system, the species are not occupying identical niches. In the first place, the system is neither closed nor constant, but fluctuates with the age of the food. The greater success of *D. melanogaster* on fresh food and of *D. funebris* on older food indicates a difference in their methods of utilizing the energy source. This difference is probably related to the differences between the micro-organisms on the fresh medium and those on the old medium, which have not yet been analyzed. The terms "fresh food" and "old food" are a simple means of referring to this undoubtedly more complex situation. Furthermore, if the food is not replenished, *D. funebris* does win out over *D. melanogaster.* Theoretically, on a constant supply of fresh food, *D. melanogaster* should eliminate *D. funebris,* but this experiment is not feasible. The food would have to be renewed so frequently that no *D. funebris* would emerge even without competition.

Since, in mixed populations, there was a delayed appearance of *D. funebris* pupae compared to their time of appearance in pure cultures, mixtures of the larvae of the two species were studied. To facilitate counting, petri dishes with a layer of yeasted medium were used. Five fertilized females of each species per petri dish were permitted to lay eggs together for a day. Controls were run for each species separately. After the adults were removed, the eggs were counted. Fortunately, the eggs of *D. melanogaster* have only two filaments while those of *D. funebris* have four. It is therefore possible to make a positive identification of the eggs laid by each species. Larval counts were not satisfactory because of the activity, the burrowing, and the lack of a simple means of classification of the larvae. However, the pupae are quite different in color and appearance so that the number of individuals of each species successfully completing the larval period could be readily determined.

Table 2 shows that, under these conditions, the presence of alien females had no apparent effect on the rate of egg deposition. The standard errors give an indication of the variability in the numbers of eggs per five females, but the sample size was also small. The *D. melanogaster* females appeared to lay eggs at a rather low rate, but it was the same in the controls as in the mixed populations.

TABLE 2

EFFECT OF ALIEN SPECIES ON EGG LAYING

	Mean eggs/5 ♀♀
D. funebris control	87.7 ± 17.4
D. funebris (with *D. melanogaster*)	100.0 ± 17.9
D. melanogaster control	34.3 ± 12.4
D. melanogaster (with *D. funebris*)	33.0 ± 1.6

Table 3 shows the results of these experiments. Only one of the controls for each species was followed through this stage. The proportion of *D. melanogaster* eggs which reached the pupal stage did not differ significantly in the pure and mixed cultures and was over 90 percent in both cases. The presence of *D. funebris* larvae had no effect on the development of the *D. melanogaster* larvae even though they outnumbered the *D. melanogaster* larvae by at least three to one.

TABLE 3

THE EFFECT OF LARVAL COMPETITION

	Control		Mixed population				Control	
	melanogaster		melanogaster		funebris		funebris	
	n	percent	n	percent	n	percent	n	percent
Eggs	24	100.0	130	100.0	445	100.0	131	100.0
Pupae	22	91.6	127	97.7	277	62.3*	124	94.7

*Difference from the control is statistically highly significant.

On the other hand, the proportion of *D. funebris* eggs reaching pupation was definitely decreased in mixed larval populations. Almost 95 percent of the eggs formed pupae in the controls, but just over 60 percent pupated when *D. melanogaster* larvae were also present. Thus, under these conditions, *D. melanogaster* had an adverse effect on the development of *D. funebris.*

Figure 2 shows something of the nature of this effect. On the seventh day after egg laying began, pupation of *D. melanogaster* larvae was complete in both pure and mixed cultures. However, the first *D. funebris* pupae appeared in the control on the eleventh day, but not until the fourteenth day in the mixed populations. Even though no *D. melanogaster* larvae were present after the seventh day, not only was the rate of development of the *D. funebris* larvae retarded, but a third of them never pupated. It can be assumed that the losses of *D. funebris* in this case occurred primarily in the larval period. If there were no larval competition, but instead the *D. melanogaster* eggs hatched first and the larvae damaged or buried a certain proportion of the *D. funebris* eggs, then the time of pupation for *D. funebris* should have been the same in both control and mixed populations. Actually, the development of *D. funebris* averaged about three days longer in the mixed populations. In addition the hatchability of *D. funebris* eggs in the presence of *D. melanogaster* eggs laid at the same time was tested directly. Although the *D. melanogaster* eggs hatched sooner than those of *D. funebris* on the average, the young larvae did not affect the hatchability of *D. funebris* eggs significantly. The range in pupation time, even in the *D. funebris* control, indicates that the medium was not the optimum environment for the development of the larvae although 94.7 percent of the eggs in the control ultimately pupated. The above results

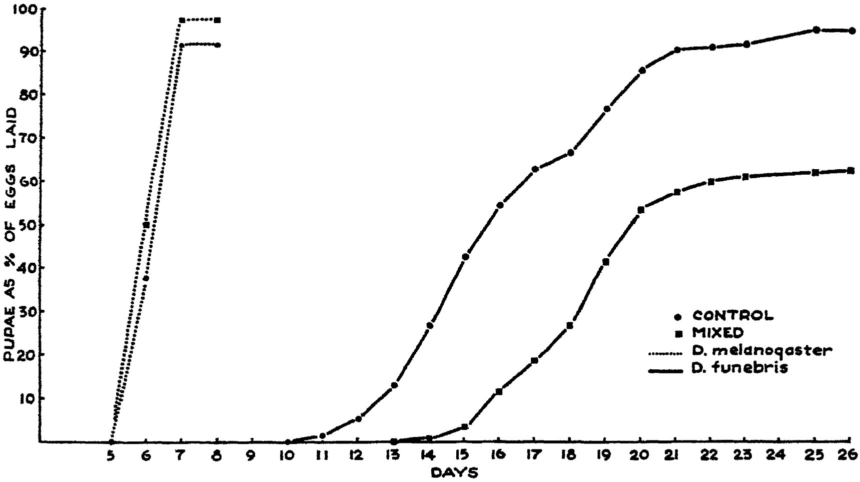

FIGURE 2. Competition between larvae of *D. melanogaster* and *D. funebris.*

with mixed larval populations show clearly that the two species do compete with one another directly to some extent.

Since the species are in direct competition, an investigation of the way in which both manage to survive was undertaken. The necessary clue lay in the fluctuations in the relative proportions of the species with the change in age of the food.

Two sets of experiments were run to test this difference. In the first, the half-pint bottles from a population unit were separated, and the larvae present were allowed to develop (table 4). The larvae were strained from the food in the old bottle and placed on fresh food. All the larvae from the old bottle turned out to be *D. funebris.* Adults of both species had had access to the new bottle for three days. These adults were removed and development allowed to proceed in the new bottle. Only *D. melanogaster* imagoes emerged. Thus, there was an apparent complete segregation, in this case, of the larvae. However, since at some time, a "new" bottle becomes "old," there must be a period when both kinds of larvae are present in the same food.

TABLE 4

LOCATION OF LARVAE IN A POPULATION UNIT

	Funebris		Melanogaster	
	♂	♀	♂	♀
Initial adult population	3	16	11	12
Adults emerging				
Old bottle	11	17	0	0
New bottle	0	0	95	107

In order to find out more about the differences between the larvae, a "Dobzhansky" population cage was started. The initial population was composed of *D. funebris*. This population was well established after two months, and 20 *D. melanogaster* were then introduced, 10 of each sex. Samples were taken from food cups of various ages to determine the proportions of each species present. The contents of a cup were transferred to two half pint bottles containing additional food so that favorable conditions for development of the larvae existed. The results are shown in table 5. Without regard to the adult population, which, of course, is a factor in the larval population, it can be seen that *D. funebris* inhabited both old and new food cups, but practically no *D. melanogaster* were obtained from the old food. In the cases in which both species were present in a cup, almost all of the *D. melanogaster* had emerged before any *D. funebris* adults appeared. *D. melanogaster* was evidently able to maintain itself in the cage because of its faster rate of development on the fresh medium. However, this initial advantage did not persist, and eventually the food was suitable only for the development of *D. funebris*. The possibility that the females differ in their choice of sites for oviposition would, of course, also influence the results. However, the interpretation given above is probably essentially correct since the course of events was similar in the mixed populations in which the females had no choice because no food was added.

TABLE 5

LARVAL POPULATION IN CAGE

Days after melano. added	Age of food	Funebris		Melanogaster		Age of food	Funebris		Melanogaster	
		n	percent	n	percent		n	percent	n	percent
6	39	164	100.0	0	0.0	26	153	100.0	0	0.0
22	42	208	99.5	1	0.5	12	167	97.1	5	2.9
	42	141	100.0	0	0.0					
36	30	441	99.8	1	0.2	10	582	70.2	247	29.8
40	30	930	100.0	0	0.0	14	884	86.6	137	13.4
76	50	94	100.0	0	0.0	17	538	82.3	116	17.7

Lin (unpublished) has performed certain experiments which throw further light on the larval competition. He studied competition between just two larvae for a quantity of yeast insufficient for the normal development of both. If both larvae were of the same species, either *D. funebris* or *D. melanogaster*, they both failed to grow normally. However, if a larva of each species was present, the *D. melanogaster* larva developed normally, but the *D. funebris* larva did not. These results confirm the superiority of *D. melanogaster* over *D. funebris* in its ability to grow on yeast.

Table 6 and figure 3 contain the data on the adult population in the cage. The build-up of the *D. melanogaster* population in the cage was quite regular until by the 73rd day practically all the adults were *D. melanogaster*.

However, by that time, all except three cups were 39 days old or more. Table 5 shows that cups of this age could not support *D. melanogaster* larvae so that the sudden change in 11 days from an adult population consisting of almost 100 per cent *D. melanogaster* to one of almost 100 per cent *D. funebris* is not surprising. The addition of food cups at regular intervals should stabilize the proportions of the two species in the population.

TABLE 6

SAMPLE OF ADULT POPULATION IN POPULATION CAGE

Day	Funebris		Melanogaster	
	n	percent	n	percent
0*	...	100.0	...	0.0
22	...	100.0	...	0.0†
42	152	98.1	3	1.9
47	125	86.8	19	13.2
51	150	75.0	50	25.0
55	158	69.7	72	31.3
61	57	37.5	95	62.5
68	28	31.8	60	68.2
73	0†	0.0	58	100.0
77	2	18.2	9	81.8
79	6	54.5	5	45.5
83	20	100.0	0†	0.0
87	13	100.0	0†	0.0
90	49	81.7	11	18.3
97	41	97.6	1	2.4
99	36	90.0	4	10.0
101	30	66.7	15	33.3
104	45	49.4	46	51.6
107	37	35.2	68	64.8
110	12	7.8	142	92.2
115	35	24.3	109	75.7
121	15	8.3	166	91.7
125	10	3.6	265	96.4

* Day *D. melanogaster* were introduced.
† Adults of this species were observed in the cage.

DISCUSSION

It may be concluded that the coexistence of *D. melanogaster* and *D. funebris* in laboratory populations does not constitute an exception to the mathematical theories of Lotka and Volterra. If the populations are run in a closed system to which no food is added, one species, *D. funebris*, does eliminate the other. However, if the populations are run in an open system to which food is added at intervals, both species are able to survive. The relative proportions of the species will depend upon the condition of the food. In this case, the survival of both types is due to the fluctuations in the environment which favor first one species and then the other. This case differs to some extent from the work with graminivorous insects (Crombie, 1947) and Protozoa (Gause, 1934) where two species coexisted

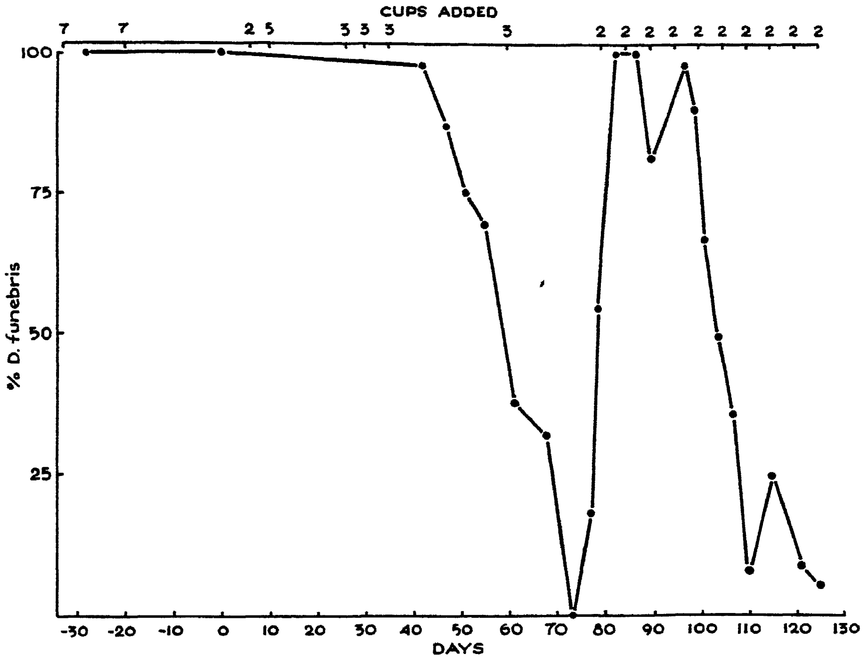

FIGURE 3. Competition between *D. melanogaster* and *D. funebris* in a population cage.

because they occupied different ecological niches. It is more comparable to the study of the polymorphic ladybird beetle, *Adalia bipunctata,* by Timofeef-Ressovsky (1940). The dark phase is favored during the summer months, but a greater proportion of the light phase survives hibernation. Thus, in that instance, the changing of the seasons favors alternate types and both survive.

Both *D. funebris* and *D. melanogaster* are cosmopolitan species whose wide distribution is evidence for their success under many conditions. *D. melanogaster* is generally regarded as a tropical species which has been introduced into many parts of its present range, but is absent from more northern regions and probably does not over-winter out-of-doors in some areas where it has been collected (Sturtevant, 1921). *D. funebris,* on the other hand, is probably primarily a temperate zone species (Timofeeff-Ressovsky, 1933) with a range extending farther to the north than that of *D. melanogaster.* Timofeeff-Ressovsky (1935) has shown that some of the success of *D. funebris* in various regions of the western Palaearctic can be attributed to the genetic adaptation of local populations to the temperatures prevailing in those regions. The work of Moore (1949a, 1949b) with the widely distributed leopard frog, *Rana pipiens,* has shown that similar adaptations make its distribution possible. It is conceivable that the success of many widespread species may be due not to some indeterminate innate superiority but to the ease with which their genotypes can be modified by selection to adapt to a wide range of environmental conditions. However, although *D. funebris* and *D. melanogaster* are cosmopolitan species, it seems clear that in a given constant environment, one species will eliminate the other even though without competition the latter might survive.

SUMMARY

1. Interspecific competition between populations of *D. melanogaster* and *D. funebris* was studied.
2. Both species coexisted in population bottles for almost two years.
3. The survival of both species does not constitute an exception to the mathematical theories of competition of Lotka and Volterra.
4. Analysis showed that most of the competition occurred between the larvae.
5. The survival of both species can be attributed to fluctuations in the environment which favored first one species and then the other. The fresh yeasted medium was more favorable to the development of *D. melanogaster* larvae. As the food aged and other microorganisms appeared, it was more suitable for the growth of *D. funebris* larvae.

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"GLIDING" IN AMPHIBIANS AND REPTILES, WITH A REMARK ON AN ARBOREAL ADAPTATION IN THE LIZARD, ANOLIS CAROLINENSIS CAROLINENSIS VOIGT.

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Thomas Belt (1874), in his interesting account of the natural history of Nicaragua, was impressed by the leap of a green lizard of the genus Anolis from a height of more than fifty feet. The lizard was being pursued by a green snake; both prey and predator came out of the tree in the same fashion. As soon as they hit the ground, the pursuit was continued without hesitation. The lizard had alighted on its feet and immediately hurried away.

The ability of arboreal forms to leap from great heights without harm is a valuable adaptation to life high above the surface of the ground. This ability reaches its maximum development among non-flying animals that have developed some form of a patagium and that are capable of gliding. Several phyletic lines are represented in this category of gliding forms. The best known examples among terrestrial vertebrates are: the Flying Squirrels (Subfamily Petauristinae), the Flying Phalangers (Genera Acrobates and Petaurus), and the Flying Lemurs (Genus Cynocephalus), among mammals; and the Flying Lizards (Genus Draco), among reptiles. A second category, less well-known, but of considerable interest, contains forms that possess small structural and behavioral modifications that enable the animals to retard the speed of descent through the air. With these forms descent is more rapid and at a lesser angle from the vertical line than is descent by gliding. In this category belong forms whose vernacular names erroneously imply a greater ability of aerial locomotion than is actually possessed; for example, the Flying Frog (several species in the Genus Rhacophorus) and the Flying Snake (Genus Chrysopelea).

These categories represent two extremely different degrees of arboreal adaptation and therefore it seems desirable to designate the two modes of descent by appropriate terms. In the past, the words "flying" and "gliding" have been used most commonly to describe aerial descent by non-flying vertebrates. The two terms have distinctly different meanings. Briefly, "flying" is aerial locomotion involving propulsion by means of the flapping or beating of wings, whereas "gliding" is aerial locomotion without any "motor" propulsion (i.e., beating of wings) wherein the animal loses altitude in order to maintain forward motion. Among living species of amphibians and reptiles there are no species that can properly be designated as *flying* forms.

Other terms, such as volplane, plane, and soar, have also been used to describe the aerial movements of amphibians and reptiles. Hesse, Allee and Schmidt (1937) speak of structural "parachutes" and of "parachuting" forms. They state, "The essential principle involved is the development of rigidly supported membranes which enlarge the under surface of the body and permit gliding flight." This includes the mammals and the lizards mentioned in the first category above but excludes the forms named under the second category. Mertens (1948) employed the term "parachute fliers" (Fallschirmflieger) for the non-flying animals that can descend through the air from great heights without injury. He included under this heading both categories mentioned above. The term, parachute, is an accurate designation for the mode of descent described in the second category above.

Despite its broad usage to describe smooth movements that may be employed in locomotion through the air, on land, in water, or on ice, the term "glide" appears preferable to similar terms that might be employed to describe the first type of aerial locomotion given above. Here the emphasis in the use of these two terms is on the mode of descent rather than on structural modifications *per se*. On a purely arbitrary basis, the two modes of descent can be differentiated on the basis of the angle that the descent path takes from the vertical line. Thus *parachuting* can be considered as the making of a retarded descent along a path that deviates from the vertical by not more than 45°, whereas *gliding* is the performing of a retarded descent along a path of more than 45° from the vertical. It is obvious that these two modes of descent will merge from one to the other, as do nearly all modes of locomotion in vertebrates. However, the two terms, as defined here, provide a convenient means of designating the differences in the degree of development of this arboreal adaptation. There are also differences in the degree of morphological specialization associated with the two modes of descent. For example, the gliding lizards (Draco) have a well-developed patagium that is supported by the elongated ribs. No such elaborately expanded and supported surface area is found in the parachuting forms.

Among amphibians and reptiles, the only forms definitely known to glide are the lizards of the genus Draco, of the tropical forests of southeastern Asia and the East Indies. The Fringed Geckos of the Genus Ptychozoon have been reported to glide but this now appears doubtful (Smith, 1935). These geckos, on the basis of the available accounts in the literature, would be referred to as parachuting forms. A number of other geckos of the Old World tropics and subtropics have probably developed this ability to some degree, for example, the Leaftailed Geckos of Madagascar (Genus Uroplates) and of Australia (Genus Phyllurus).

The only other recent reptiles that have been reported to show such adaptation are the tree snakes of the genera Chrysopelea and Dendrelaphis. The former of these includes the so-called "Flying Snakes." The mode of descent observed in these is parachuting. This is accomplished by the snake leaping from a high limb, stretching out lengthwise, flattening and broadening the body, while drawing in the belly so that it forms a concave surface. In this manner these snakes have been observed to descend slowly and at an angle to the vertical.

Among amphibians only frogs have been reported to take to the air from great heights. Alfred Russel Wallace (1869) is responsible for one of the earliest reports on the parachuting ability of some members of the Genus Rhacophorus of the Indo-Malayan region. Several arboreal species of this Genus (*nigropalmatus* and *reinwardtii*) possess enormous webs between the fingers and toes, making a large expanse of skin surface relative to body weight. The aerial ability of these frogs has been much discussed. Ayyanger (1915) reported these frogs to be excellent gliders, whereas Barbour (1926) maintained that the ability of the frogs is greatly exaggerated. There seems to be little doubt that they are parachuters, rather than gliders, as the terms have been redefined here.

In Brazil, Cott (1926) performed some experiments that demonstrated a well-developed parachuting ability in a local tree frog, *Hyla venulosa*. When dropped from a height of 140 feet the frog landed ninty feet away from the vertical line! Cott later performed some experiments to evaluate the assertion that any small frog could probably duplicate this parachuting descent because of the greater surface area relative to body weight. He used a common terrestrial frog of Europe (*Rana temporaria*) and a European tree frog (*Hyla arborea*). The frogs were selected to approximate the size and weight of the Brazilian frogs. Both European species fell vertically and heavily. Moreover, there was an important behavioral difference between the Brazilian and the European frogs in falling. When the Brazilian frogs were launched into the air, they always stretched the limbs out at the sides in a constant and characteristic attitude, with the belly downward, even when tossed into the air in such a fashion that they turned over and over. Both European frogs turned over and over during the fall, and did not assume any constant position. It was thus purely chance whether they alighted on their feet or on their backs.

Mertens (1948) points out that the ability to glide or parachute is far more common among animals of the tropical rainforest than it is in those of any other habitat. This is especially true for amphibians and reptiles, and is, perhaps, to be expected in view of the greater numbers of arboreal species that occur in the rainforest as compared to none or only a few arboreal forms elsewhere. Certainly the most pronounced arboreal specializations to be found in amphibians and reptiles are present in the species of the tropical and subtropical areas: for example, the presence of a prehensile tail, adhesive disks, and stereoscopic vision.

Except for Cott's Brazilian Hyla, the only amphibians and reptiles that definitely have been reported to glide or parachute are inhabitants of the Old World tropics. It is to be expected that additional species inhabiting both the Old and the New World will be added to the list of forms now known to parachute.

A PARACHUTING LIZARD

One of the previously unreported parachuting forms is the Carolina Anole, *Anolis carolinensis carolinensis* Voigt. This lizard has well-developed, expanded adhesive pads on the fingers and toes. With the aid of Edwin H. McConkey, I performed a series of simple experiments, to test the presence or absence of any ability to parachute in this lizard. Four lizards of approximately the same weight and size were dropped from a height of thirty-seven feet, two inches. They alighted six, six, ten and fifteen feet distant from the vertical line. The day was sunny and mild, with virtually no wind. The air temperature was 29°C, only slightly below the eccritic temperature of this species, 31.4°C.

One of the most interesting observations made in the experiments was that, in falling, all the specimens assumed a constant and characteristic posture with the limbs outstretched and the belly downward. Once this posture was assumed, there was no noticeable movement except for a slight wiggling of the tail. All the lizards fell vertically for one-third to one-half of the distance and then suddenly veered away from the vertical before striking the ground. In one instance the lizard alighted on concrete but was unharmed. From these experiments, the Carolina Anole seems to show a marked ability to parachute.

To obtain some comparative observations on another partly arboreal lizard of this same region, three Southern Fence Lizards, *Sceloporus undulatus undulatus,* were dropped from the same height and on the same day. These lizards were selected to approximate the weight or the length of the Anoles, but the species is much stockier and heavier. Two of the Fence Lizards were of the same weight as the Anoles but shorter; the third individual was of equal length but much heavier. Regardless of size, all the Fence Lizards alighted no farther from the vertical line than two feet and appeared to fall harder than the Anoles. The most striking difference noted was the fact that the Fence Lizards wiggled violently all the way down and did not assume any constant and characteristic posture; however, they appeared to alight on their feet.

In contrast to the Anole, the Fence Lizard does not appear to have a well-developed ability to parachute. Both species inhabit trees and bushes, at least part of the time. The Anole appears to be more arboreal than the Fence Lizard and is more frequently found at greater heights from the ground. The difference in ability to parachute in these two forms is probably due in large degree to the morphological differences and overall size. The proportion of surface area to body mass is considerably greater in the Anole than it is in the Fence Lizard. One of the Anoles had a snout-vent length of 65 mm and weighed 6.4 grams; a Fence Lizard of the same length weighed 15 grams. Fence Lizards lack expanded adhesive pads on the fingers and toes.

The Fence Lizards made no effort to assume and maintain while falling a constant posture with the arms and legs outstretched. This behavior was noticeably different from that of the Anoles in which a characteristic posture was assumed by all of the individuals observed. This posture, with arms and legs outstretched and the belly downward, exposes the maximum possible surface, including the area of the expanded adhesive pads. It is of interest to note that the posture assumed by the Anoles is very similar to that reported by Cott for the Brazilian Hyla and for the parachuting Rhacophorus (see Cott, 1926, figs. 1-3). This is approximately the same position that is assumed by the gliding lizards (Draco), and the gliding mammals when in the air. Since these experiments, I have observed the same phenomenon in wild lizards under natural conditions. On several instances when I have pursued wild Anoles to the top of small trees they have endeavored to escape by leaping to the ground. Each time the lizard assumed the characteristic posture when in the air.

The evolution of this behavioral pattern in arboreal species appears to be of the utmost importance and is a constant characteristic of those forms that are known definitely to parachute. In the evolutionary development of the ability to parachute it appears that the acquisition of the behavioral pattern has proceeded independently of, and probably subsequent to, the development of structural modifications acquired for that purpose. All parachuting forms mentioned here differ considerably in their morphological attributes yet exhibit the same basic behavior pattern when parachuting. The morphological differences between the Brazilian *Hyla venulosa* and the European *Hyla arborea* are not sufficient to account for the vastly different performance recorded in Cott's experiments. Here the difference in performance results from a difference in behavior under the same circumstances.

It would be of considerable value and interest to study the differences in the ability to parachute in different species of a large genus containing arboreal and semiarboreal species in order to analyze the evolutionary development of the adaptive behavior patterns and various morphological adaptations associated with the arboreal habit. Among amphibians and reptiles, frogs of the genus Hyla and lizards of the genus Anolis would provide excellent material for such an investigation. In each of these genera there are highly specialized arboreal species, semiarboreal species, and terrestrial species. These differences in habits appear to be associated with well-marked differences in morphology and behavior, at least in the genus Anolis (Oliver, 1948). However, the actual analysis of these in relation to one another and in relation to phylogeny should prove a fascinating study and would considerably increase our understanding of the evolution of adaptive attributes.

SUMMARY

The ability of some amphibians and reptiles to descend through the air from great heights without harm is reviewed. Among living species, two extremes of development of this arboreal adaptation are recognized on the basis of two different modes of descent: gliding and parachuting. The ability to parachute is reported for the Carolina Anole, *Anolis carolinensis carolinensis* Voigt.

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EFFECT OF TEMPERATURE AND MOISTURE CONTENT OF THE NUTRIENT MEDIUM ON THE VIABILITY OF CHROMOSOMAL TYPES IN DROSOPHILA PSEUDOOBSCURA

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Most natural populations of *Drosophila pseudoobscura* are known to be mixtures of chromosomal types which differ in the gene arrangements in the third chromosomes. Observations in nature as well as laboratory experiments have shown that these chromosomal types differ in their adaptive values in different environments. Thus, at the temperature of 25°C, the heterozygotes for the Standard and Chiricahua gene arrangements are superior to the Standard homozygotes, and the latter are superior to the Chiricahua homozygotes. And yet, at 16°C the adaptive values of these chromosomal types are alike within the limits of the experimental errors (Dobzhansky, 1947). The influence of the humidity of the air on the survival rates of pupae, and of temperature on the longevity of the adult flies have been studied by Heuts (1947, 1948). The experiments reported below suggest that the moisture content in the culture medium in which the larvae develop also acts differentially on the survival of the different chromosomal types.

The material used consisted of eight strains, each homozygous for Standard, Arrowhead, or Chiricahua gene arrangements. The progenitors of these strains were collected at Pinon Flats, Mount San Jacinto, California, in 1944. These are the same strains which were used in the experiments of Dobzhansky and Heuts. Standard, Arrowhead, or Chiricahua flies were outcrossed to a laboratory strain which carried in its third chromosome the dominant gene Lobed and the recessive orange. The gene arrangement in the third chromosome marked with the gene Lobed was Santa Cruz; very little detectable crossing over takes place in the heterozygotes carrying Santa Cruz and the other chromosomes. In the F_1 generations of these crosses, Lobed/Standard, Lobed/Chiricahua, and Lobed/Arrowhead flies were selected and inbred in different culture bottles. Since Lobed is lethal when homozygous, a segregation in the ratio of 66.67 per cent Lobed: 33.33 per cent wild-type (homozygous for Standard, Arrowhead, or Chiricahua chromosomes respectively) should occur. Since, however, the gene Lobed reduces the viability of its carriers, the ratios actually observed vary, depending upon the viability of the different homozygous chromosomal types. Observations on these ratios permit, therefore, comparisons of the viability performance of the Standard, Arrowhead, and Chiricahua homozygotes.

Two kinds of nutrient media have been used in the experiments. One of them, denoted as "Regular," has been described by Spassky (1943). It consists of 500 gm. of cream of wheat and 533 cc. of molasses in 3600 cc. of water. The "Dry" medium contained 500 gm. of cream of wheat and 400 cc. of molasses in 1800 cc. of water. Samples of both media were analyzed for water content. The regular medium contained about 72 per cent, and the dry medium about 54 per cent water.

Fifteen Lobed females and a like number of males served as parents of each culture. In all cases the females and males came from different strains with the same gene arrangement. This precaution is very important. Indeed we are interested in the relative viabilities of the Standard, Arrowhead, and Chiricahua flies as classes, and not in the viability of homozygotes for individual chromosomes, which may contain recessive viability modifiers, including lethals. The flies were permitted to oviposit for about twenty-four hours at room temperature, whereupon the adults were transferred, without etherization, into new oviposition bottles. The bottles with the eggs deposited in them were placed in constant temperature rooms either at 25°C or at 16°C. When the F_2 generation hatched, counts of Lobed and wild-type flies were made at 3-day intervals, until the cultures were exhausted. The viability data are summarized in table I.

No significant differences in frequencies of wild-type flies have been found among the homozygotes for the different gene arrangements developing on the normal medium at 25°C. At the same temperature, but on the dry medium the Chiricahua homozygotes survive relatively better than do Standard or Arrowhead homozygotes (the difference between Chiricahua and Standard is 3.44 ± 1.62 per cent in favor of Chiricahua, and that between Chiricahua and Arrowhead 6.6 ± 1.46 per cent in favor of Chiricahua. At 16°C, the only significant difference seems to be that between Standard and Arrowhead on regular food (3.63 ± 1.72 per cent in favor of Arrowhead).

TABLE I

PERCENTAGES OF WILD-TYPE FLIES IN CULTURES WITH DIFFERENT GENE ARRANGEMENTS

Gene arrangement	Regular medium		Dry medium	
	Flies counted	Per cent wild-type	Flies counted	Per cent wild-type
Standard 25	1031	39.18 ± 1.54	1557	37.44 ± 1.23
Chiricahua 25	1313	36.10 ± 1.32	2096	40.88 ± 1.07
Arrowhead 25	923	35.31 ± 1.57	2258	34.72 ± 1.00
Standard 16	1454	35.49 ± 1.25	1597	36.01 ± 1.20
Chiricahua 16	2745	37.38 ± 0.94	2844	34.38 ± 0.89
Arrowhead 16	1654	39.12 ± 1.20	2589	34.80 ± 0.94

More interesting for our purpose are differences in the viabilities of the same chromosomal types in different environments. Chiricahua homozygotes are relatively more viable on the dry medium than on the regular medium at 25°C (the difference is 4.78 ± 1.70 per cent). At 16°C Chiricahua homozygotes are more viable on regular than on the dry medium, the difference being 3.00 ± 1.28. Arrowhead homozygotes are more viable on the regular than on the dry medium at 16°C (the difference is 4.32 ± 1.52 per cent), although apparently not at 25°C. The Chiricahua homozygotes are relatively more viable at 25°C than at 16°C when raised on the dry medium (the difference is 6.50 ± 1.39 per cent), although no such difference is apparent on the regular medium.

It should be kept in mind that the present study is concerned with comparisons of the relative viabilities of the different chromosomal types during only a part of the life cycle, extending from the egg stage to the hatching of the adult. The viability, the fecundity, longevity, and other physiological characteristics of the adult flies are beyond the scope of the present study. The bearing of the present findings on the situation observed in natural and in experimental populations of *Drosophila pseudoobscura* is therefore limited. However, the characteristics studied are components of the overall adaptive values of the chromosomal types compared. As far as our data go, Chiricahua homozygotes seem to have certain survival advantages in warm and dry environments. It may be interesting in this connection to recall that the Chiricahua gene arrangement reaches its highest frequencies in the populations of Northern Mexico (Dobzhansky, 1948). On the contrary, the Arrowhead homozygotes may be superior in colder and more humid environment. The geographic distribution shows the highest frequencies of Arrowhead in the Great Basin, northwards up to the interior of British Columbia.[1]

SUMMARY

The relative viabilities of the homozygotes for the Standard, Arrowhead, and Chiricahua gene arrangements have been compared in cultures with regular and with dry nutrient medium. The cultures were kept at temperatures of 16°C and 25°C. Chiricahua homozygotes find optimal conditions on the dry food and at 25°C, while Arrowhead homozygotes are superior on the regular food and at 16°C.

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[1] I wish to express my gratitude to Dr. Th. Dobzhansky for constructive suggestions.

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A LIST OF CHEMICAL SUBSTANCES STUDIED FOR EFFECTS ON DROSOPHILA, WITH A BIBLIOGRAPHY[1]

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It was not until recently that the induction of heritable changes by chemical substances was clearly demonstrated in experiments with Drosophila by Auerbach and her associates. However, earlier work on the genotypic and phenotypic effects of various chemicals on Drosophila contain a wealth of information which may be of use to the investigator. There are several studies on chemically induced mutation which are not widely known, particularly those done in Russia by Rapoport, Gershenson, and others. Therefore, it was felt that a compilation of titles and a list of chemicals studied thus far in Drosophila would be useful.

The present handlist contains 314 references, including titles through the year 1950. Except for 50 new references, this work is almost identical with the one included by Dr. M. Demerec in Drosophila Information Service, No. 24: 107–122, 1950. In general the bibliography does not list papers concerned solely with ionizing and non-ionizing radiations, temperature, or age. A number of papers dealing with the lethal effect of insecticides on Drosophila have also been omitted. However, a few titles are included that show the variety of methods by which the induction of mutations has been attempted. References to studies on the development of a chemically defined medium for Drosophila are included since it is becoming apparent that more meaningful results will be obtained when the food variable is controlled.

The review articles by Timoféeff-Ressovsky (233) and Auerbach (10,11) have not been cross-indexed with the list of chemical substances. An asterisk in the list of chemical substances means that no test for mutagenicity has yet been made. Numbers following each substance refer to entries in the bibliography. An asterisk in the bibliography indicates that the reference contains no work on chemical mutagens; a question mark means that the paper was not available for checking.

CHEMICAL SUBSTANCES

Acetic acid—142, 144, 172, 260
Acetylaminofluorine—64, 67, 74, 75
Acenaphthene—264

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[1] This work was initiated at the Biological Laboratory, Cold Spring Harbor, N. Y., and completed in the Department of Surgery, L. S. U. School of Medicine where it has been supported by a grant from the National Cancer Institute, U. S. Public Health Service.

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D. I. S. = Drosophila Information Service

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DELETERIOUS GENES IN WILD DROSOPHILA MELANOGASTER FROM ISRAEL[1]

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Since the concentration of unfavorable recessive genes in populations may furnish a clue to their hidden variability on the whole, and to their structure, size and breeding habits, it is important to gather a maximum of information on deleterious genes in populations of different geographic origin in a variety of species. Comprehensive investigations of lethal gene content in *D. pseudoobscura* were carried out by Dobzhansky and co-workers (1938, 1941, 1942). Recently, Brazilian populations of *D. willistoni* have been subjected to a similar analysis by Pavan et al. (1951). For a study of the variation in concentration of lethals under different environmental conditions, the cosmopolitan *D. melanogaster* should be a suitable object, but for this species only two sets of data from widely distant regions are available at present. While Ives's (1945) results from the United States indicate a very high concentration of lethal and semilethal genes (34–65 percent), Dubinin's (1945) figures for Russian populations are consistently lower, ranging from 9.8–29.2 percent, with the single exception of the Kutaisi population, which in November 1939, contained 38.9 percent lethals. Unfortunately, the results of these two authors are not strictly comparable, for it is not known to what level of viability semilethals are included in Dubinin's data.

However this may be, the population structure in the two areas investigated must be very different and it seems desirable to gain some information on the hidden variability of this species in other climatic regions. As a first contribution to an analysis of Middle East *D. melanogaster* populations, 243 second chromosomes from Israel were investigated by backcrossing to the *Cy L/Pm* stock, a modified 'Cl B method', which was described in detail by Wallace (1950a). Except for the few cultures, where not enough flies were available, five males and five females heterozygous for the same chromosome II and for the Cy L chromosome were utilized in the crucial F_2 cross. These five pairs were transferred twice, at intervals of three days, and the three bottles for each chromosome yielded an average of 552 flies, each bottle being scored at intervals of three days, three times, as a rule, until the appearance of the next generation. Figure 1 shows the frequencies of chromosomes of different viabilities, the latter being expressed as the percentage of homozygous wild flies appearing in the total count for each chromosome. Disregarding crossing-over, the expected viability of the average heterozygote for two viable wild chromosomes II should be 33.33 percent, provided that the viability of the *Cy L* chromosome were normal. In reality, the viability of the *Cy L* chromosome utilized is reduced by somewhat less than one percent. The crossovers, on the other hand, which are more frequent among the offspring of young females and fall off to zero as the mothers grow older (cf. Whittinghill and Hinton, 1950), in their turn amount to a little below one percent. It is therefore most convenient to pool the crossover types with the curly-lobe class, and to determine the percentage of wild type homozygotes in the total, accepting 33.33 as the standard viability of the 'normal' heterozygote.[2] Accordingly, chromosomes of a viability less than 16.67 percent in the homozygous condition should be considered as semilethal. These figures compare well with those of Ives, who assumed the theoretical 33.3 percent for the average heterozygote and fixed 17 percent as the upper limit of the semilethal range.

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The distribution of frequencies in figure 1 follows the characteristic pattern in showing two maxima, one at the lethal level (0–3.33 percent), and another one below the accepted normal level of 33.33 percent. This clustering of mutations at the lethal and at the subvital level and their comparative rarity throughout the intermediate range has been observed in all populations analyzed, wild and experimental, but developmental genetics has not furnished, as yet, a satisfactory explanation for this peculiar phenomenon.

The average viability of all chromosomes above the semilethal range amounts to 27.98 percent in the present sample. It is by no means surprising to find the average homozygote so much (5.35 percent) inferior to the average heterozygote and all workers concerned with recessive deleterious genes have considered this characteristic shift of the maximum as an indication of the importance of heterosis in the maintenance of populations.

The present analysis yielded 25.10 percent lethals (0–1.67 percent viability, corresponding to Pavan and co-workers' 5 percent viability level) and 13.58 percent semilethals, resulting in a total of 38.68 percent deleterious chromosomes. The lethals thus represent 64.89 percent of the total deleterious class. This proportion is in good agreement with Pavan and coworkers' data for *D. willistoni*, being intermediate between their percentage for the third (61.32 percent) and for the second (69.42 percent) chromosome, but it differs strikingly from the results of Ives (1945), who obtained 86.9 percent true lethals among his invisible deleterious genes, although he includes in the lethal class only those of complete inviability.

The sample from Israel analyzed here includes 41 chromosomes collected at different times in various parts of the country. The remaining 202 chromo-

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[2] Dr. B. Wallace, Cold Spring Harbor, kindly informs me that this is the average viability of the heterozygote in several series of test crosses performed in his laboratory.

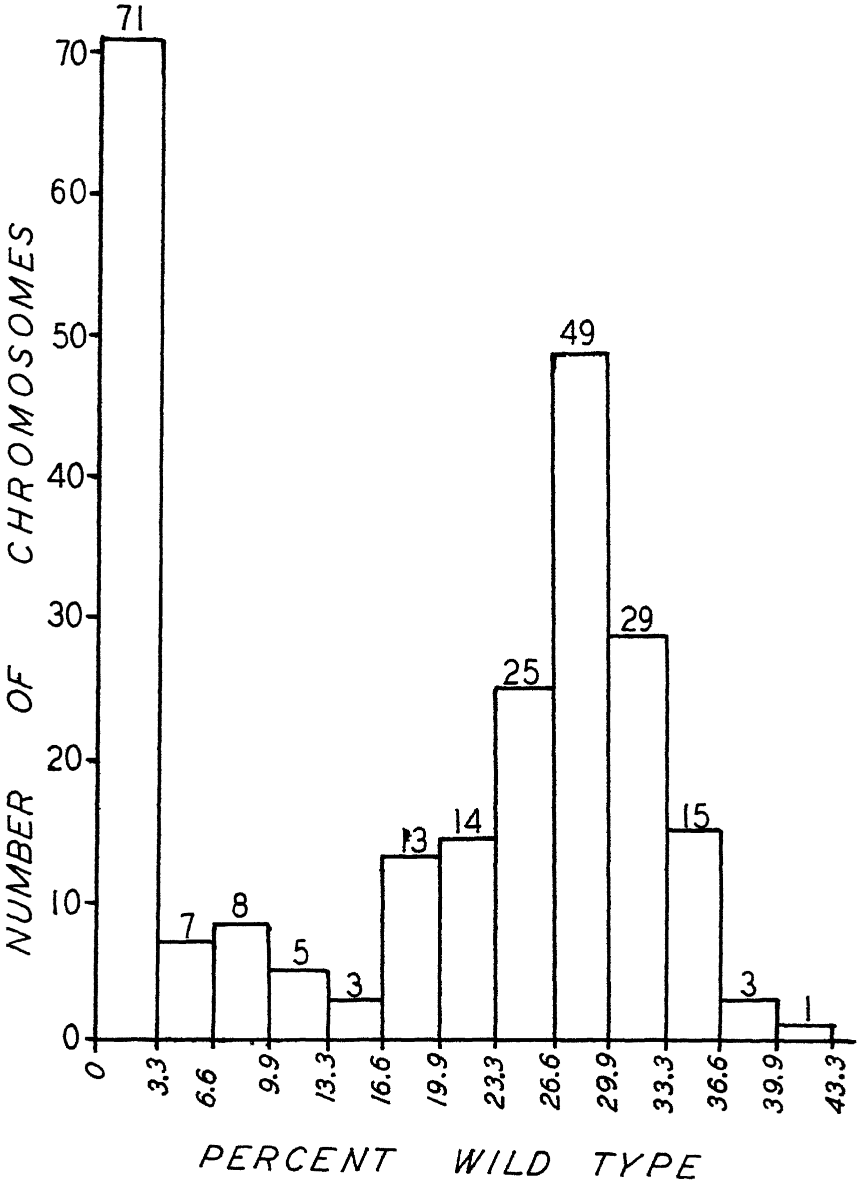

FIGURE 1. The frequencies of chromosomes of different viability classes in the total sample of 243.

somes came from two collections made by Mr. J. Wahrman in the mountainous Jerusalem area on October 20 and November 13, 1950, and may be considered to constitute a fairly uniform sample with regard to time and locality. The percentage of lethals and semilethals among these 202 chromosomes was 41.09 percent, a little above the 38.68 percent obtained for the total 243 chromosomes. This concentration of deleterious genes is comparable with the lowest figures obtained by Ives for the United States, being approximately midway between his value for Massachusetts, 1938 (45.0 percent), and that for Maine 1942 (34.2 percent). Ives's value for the viability of the average homozygote shows a certain inverse relationship to deleterious gene content; hence, it is interesting to note that the value here obtained is a little below his highest (28.53), indicating that population size at this range may be similar.

On the other hand, the percentage of deleterious genes in the Israel population of *D. melanogaster* is much higher than any of the values recorded by Dubinin for different Russian populations, with the exception of the Kutaisi population (38.8 percent). As stated above, we do not know what Dubinin defines as 'lethals,' but, assuming that he includes semilethals at least up to the 6.67 percent level, the comparable figures would be 32.1 percent for the total sample from Israel and 33.7 percent for the Jerusalem population in autumn and would still exceed all of Dubinin's percentages, except for Kutaisi.

This comparison indicates that the breeding populations of *D. melanogaster* in Israel may be fairly large, approaching the conditions reached in certain areas of the Northeastern United States. In view of the climatic peculiarities of the Middle East, where the cold rainy season is followed by a long, hot and dry summer, and in view of the difference between the colder and drier mountain climate and the hotter and more humid conditions of the coastal plain, it would be desirable to gain more specific information on the lethal concentration in different parts of the country during the seasonal cycle. While it stands to reason that the 'bottleneck' in population size in the Eastern United States should be at the close of the humid summer, it is not easy to predict when it should occur in a much more arid climate.

It is a pleasure to acknowledge the aid and encouragement received from Professor Th. Dobzhansky in the course of this study, as well as the hospitality of his laboratory.

SUMMARY

An analysis of deleterious gene content in a sample of 243 second chromosomes of wild *D. melanogaster* from various parts of Israel yielded 38.68 percent lethal and semilethal genes; 202 chromosomes out of this total were collected in autumn in the Jerusalem area, and for this more uniform sample the content of lethals and semilethals was 41.09 percent. This concentration of deleterious recessives exceeds most of the values recorded by Dubinin for Russia, and is similar to his highest exceptional value; it

ranges among the lower percentages observed by Ives in populations of the Eastern United States. Hence in spite of the semi-arid climate, the size of the breeding populations of *D. melanogaster* in Israel may be comparable to that of certain areas in the humid Eastern United States.

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NATURAL HISTORY AND THE BIOLOGICAL SCIENCES

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The partially fire-proof and calamity-proof structure that houses workers in biology today has undergone a vast amount of remodeling. Since its first appearance as a tiny cyclone cellar which the blasts of dogma could not entirely erase, it has risen into the open quite in conformity with the heliotropic movement prevailing among those forms of animal and plant life that compete in the open. Its architecture has been kept continuously modern, for its time, and consequently much has been torn away, replaced—sometimes later to be resurrected—leaving here and there a few relics of its previous form. Of course, most of these have been polished, revarnished, or otherwise superficially disguised to suit the fleeting fancy of successive tenants.

Meanwhile, as in a pioneer New England dwelling, additional ells have been found necessary for the increasing family, including marital supplements—chemists, physicists, mathematicians, sociologists. The first generation crosses of these, all evincing the expected heterosis or hybrid vigor, include biochemists, endocrinologists, biophysicists, ecologists, geneticists, eugenicists, statisticians, to mention only a few conspicuous examples.

In later generations, naturally all these varied genes have popped up in many unexpected combinations. Certain polyploid agglomerations like the anthropologists confine their attention strictly to a single species of animal, though they allow nothing pertaining to his corporeal and spiritual body, nor his past history and unpredictable future, to escape their zealous eyes. Further afield, sociologists brazenly envision the rising tide of human depravity overwhelming man as a social animal, something for which he was not modeled by Nature. No less timid souls still further transcend the biological level. They seek by force of law to induce mutations in our instinctive and innate intellectual behavior to eliminate its unsocial traits. Perhaps entomologists may be to blame for this thesis, as they have surmised with good reason that significant mutations in instinct occur at long intervals among insects.

In contrast, other biological offspring, like the general physiologists, seek to compress into a few fundamental principles all the varied activities of the innumerable world of animate nature.

Thus, workers in one specialized field of biological science at the present day recognize the devotees of another with difficulty on the street, or in the reports of their achievements before learned gatherings. These divergences began slowly, but the pace has been stepped up and the field of biology has been so unexpectedly widened and intensively cultivated in many circumscribed areas that the young naturalist who entered professional life half a century ago has seen the whole face of biological science change so completely that he can no longer see the forest for the trees. Furthermore, he is sufficiently familiar with so few of the trees thathe must be content to understand the remainder but vaguely on the authority of some colleague to whom the other denizens of the forest are equally intriguing, though unfortunately utterly strange.

This narrowness of understanding is the inevitable consequence of the biological structure itself. Those who have watched its modernization over an extended period of time have come to a gracious acceptance of their incompetence as mediators. They remain as outmoded experts in some rapidly narrowing corridor, frantically trying to keep track of their co-workers through the medium of some expansive rogues' gallery, "Who's Who," or by way of some short-cut to omniscience such as the *Biological Abstracts,* the *Zoological Record, or* other less encyclopedic digests.

Among the first groups of biologists to develop clannish propensities were the entomologists. They established numerous societies and journals that flourished during the last century, more luxuriantly after 1875. While this was going on, other zoologists continued collectively to pour their rapidly increasing output into a few more general journals, *Zeitschrifte* and *Jahrbücher.* When these finally blew up from internal pressure, the fragments congealed into an assortment of *Abteilungen* or separately named journals, variously devoted to more limited and discrete fields of research. To these entomologists are now contributing more and more extensively, together with others who find that insects are eminently suitable materials for varied kinds of experimental investigation.

With this background, it is refreshing to discern here and there on the far horizon some lone "naturalist" who still clings to the precarious supposition that the divergent phases of natural history are amenable to integration without loss of clarity or dignity. The exposition of such a viewpoint is contained in a recent book, entitled "The Nature of Natural History,"[1] by Marston Bates, an entomologist of wide and varied experience. This remarkably sane and well-balanced account of the varied aspects of natural history has furnished the occasion for the present remarks. Much that it details is commonplace to the professional biologist, but the vigorous exposition of natural history as a comprehensive background for the understanding of the several more restricted biological fields may be read universally and digested with profit. Such an outlook can do much to mitigate the insidious myopia that is creeping into teaching and research in the biological sciences.

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[1] 309 pp. Charles Scribner's Sons, New York, 1950.

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GENETIC CHANGES IN POPULATIONS UNDER IRRADIATION[1]

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The precipitate advent of the atomic age has evoked a deluge of articles concerning the biological harm resulting from radiations; geneticists, physicists, and medical scientists have all contributed their views. While the chief concern has been the immediate physiological effects of the radiation, there has been a tendency to speculate on the long-range effects which genetic changes induced by radiation will have on human populations. These speculations have ranged from badly reasoned, alarming statements in popular magazines to impressive (although not necessarily less alarming) arguments marshalled by competent scientists, including Haldane (1947), Muller (1948, 1950, a, b, and c), Evans (1949), and Wright (1950). Although these arguments are well organized, the divergent conclusions emphasize two points: the need for more knowledge, and, until that is available, the need for caution in exposing individuals to radiation.

In general, two classes of data may be gathered to further our knowledge of the genetic effects of radiation on populations. Analyses can be made of separate gene loci in order to determine mutation rates, degrees of semi-dominance, relative frequencies of mutant alleles with differing degrees of subvitality, the time of action of these deleterious alleles, and the role radiation plays in altering these factors. Using this knowledge, and the mathematics of population genetics, equilibria could be predicted for various classes of mutant genes and the relative importance of these classes in the total effect exerted by mutant genes on the well-being or adaptive value of a population.

A second general approach to the problem of irradiated populations, the one discussed in this paper, is the study of the population itself. The drawbacks of this method are that individual mutations are not followed, that the data reflect mass changes within the population, and that analyses can be made only on simplified assumptions. Nevertheless, the advantages of this approach are several: 1. The information obtained is what is wanted for practical purposes. 2. This information must be available for evaluating the results obtained by substituting figures for individual gene mutation data in the formulas of theoretical population genetics. (That the use of these general formulae will not be simple is indicated by this statement of

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[1] The work reported here was done under Contract No. AT-(30-1)-557, U. S. Atomic Energy Commission.

Wright's (1932, p. 155): "...the selection coefficient of a particular gene is really a function not only of the relative frequencies and momentary selection coefficients of its different allelomorphs but also of the entire system of frequencies and selection coefficients of non-allelomorphs.") 3. Data obtained from populations may disclose evolutionary changes evoked in response to radiation; these changes cannot be considered in the gene mutation studies, for the latter are essentially static.

The purpose of the present paper is to outline the basic postulates of the research project, to present typical data, and to discuss some of the implications of these data. The assistance of Carol V. Madden, Louis Forgione, Howard Monsees, Gloria Cosillo and Henry Gardner is gratefully acknowledged. Frequent discussions with Professor Th. Dobzhansky of Columbia University have been a continual source of inspiration. Drs. A. E. Brandt and Howard Levene of the U. S. Atomic Energy Commission and Columbia University, respectively, have given helpful suggestions concerning the handling of statistical problems.

MATERIAL AND METHODS

Six populations of *Drosophila melanogaster* were studied in order to determine the effect of radiation on populations. The analyses were concerned primarily with the second chromosome, one of the two large autosomes of this species. The original parental flies of each population carried second chromosomes that were free of lethals, semilethals, and easily detectable subvitals.

The breeding system used to test individual chromosomes in the homozygous condition is illustrated in fig. 1. Egg samples were taken from the populations at two-week intervals (or multiples thereof) and were subdivided among several cultures in order to minimize larval competition. Males (P_1) which hatched from these eggs were mated individually with *CyL/Pm* virgin females. (*CyL* designates a balancer chromosome, $Cy\ al^2\ lt^3\ L^4\ sp^2$, that carries a crossover suppressor in each limb and the two dominant genes *Curly* and *Lobe*. *Pm* refers to another second chromosome marked with the dominant gene *Plum* and which is, in turn, a convenient balancer for *CyL*.) One *CyL*/+ (F_1) male was picked from each P_1 culture and was remated with *CyL/Pm* females. The *CyL*/+ male and female (F_2) offspring of this mating carried identical wild-type second chromosomes and were inbred to obtain an F_3 which gave, if the tested chromosome carried no deleterious genes, *CyL*/+ and +/+ flies in a ratio of approximately 2 : 1. If the tested chromosome carried a lethal, only *CyL*/+ flies appeared in the F_3 culture; if the tested chromosome carried a semilethal, the 2 : 1 ratio was distorted in favor of *CyL*/+. Since genes that affect viability do not fall into sharply defined classes, we adopted the following conventions: a lethal was defined as a chromosome which permitted 0%–3.1% homozygous wild type (non Curly-Lobe) individuals to emerge in an F_3 culture; a semilethal, 3.2%–15.8%; and a "normal," 15.9% or more. If a culture contained 50% or more wild-type flies, it was discarded; this happened very rarely.

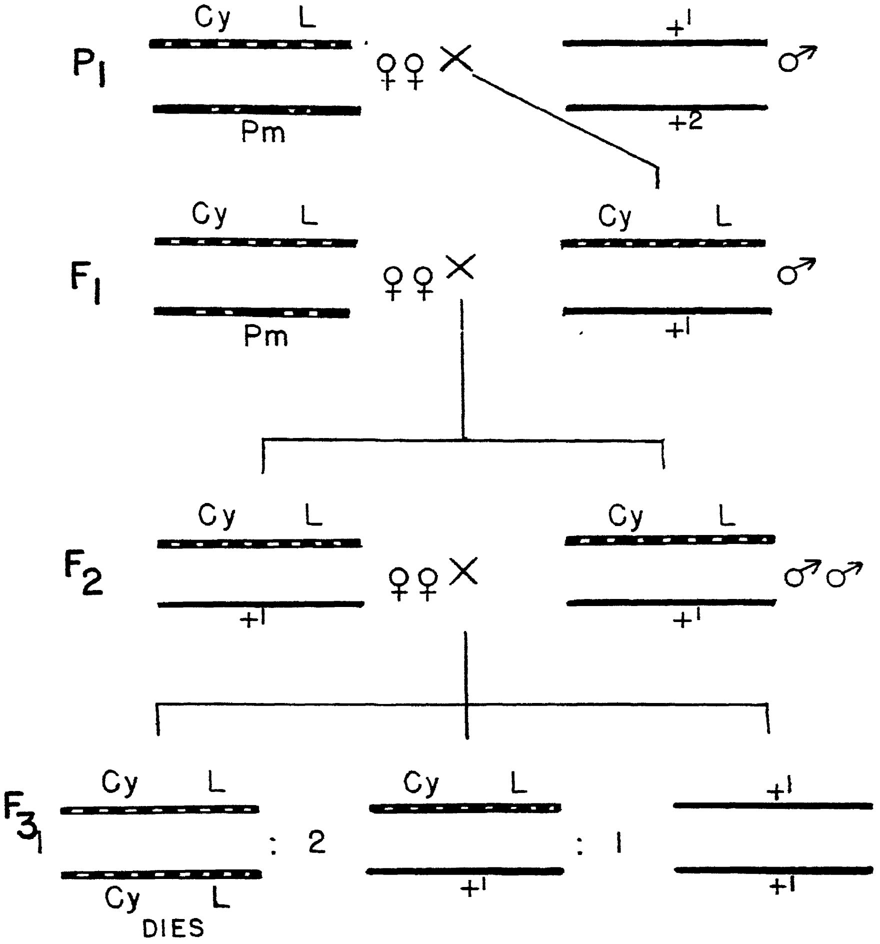

FIGURE 1. Mating system for the detection of recessive, second-chromosome genes.

Summarized descriptions of the populations and the experimental conditions are given in table 1. All populations were kept at 25°C. The sources of chronic gamma radiation were two radium bombs approximately 70 mg and 500 mg in size. All populations except #5 were maintained on cornmeal-molasses-agar medium enriched with brewer's yeast; these populations consisted of approximately 10,000 adults. Population 5 was a small population of 100–1000 adults that was maintained on un-enriched medium. The populations exposed to chronic gamma-radiation were kept in semi-circular lucite and screen population cages; these cages have been described previously (Wallace, 1949). The populations not exposed to chronic radiation were kept in oblong lucite and screen cages 18" long, × 5½" wide, × 4½" high.

TABLE 1

DETAILS OF THE EXPERIMENTAL POPULATIONS

Population	Size	Treatment	Dose	Started	Stopped
1	Large	Acute	♂♂ 7000r ♀♀ 1000r	7/25/49	...
2	Large	Acute	♂♂ 1000r ♀♀ 1000r	7/25/49	1/12/50
3	Large	None	...	7/25/49	...
4	....Special population for determining mutation rates....				
5	Small	Chronic	5.1 r/hr	4/ 1/50	...
6	Large	Chronic	5.1 r/hr	4/15/50	...
7	Large	Chronic	.9 r/hr	4/15/50	...

EXPERIMENTAL RESULTS

The data that were obtained from the populations were of two types—those revealing the chromosomal content of the populations and those indicating the condition of the populations themselves.

The changes in frequency of lethal chromosomes in populations 1, 3, 5, 6, and 7 (population 2 was discontinued after 12 generations and will be left out of this discussion) are given in table 2 and shown graphically for the four latter populations in fig. 2. It is obvious that, except for the initial decrease in the frequency of lethals in population 1, lethals accumulated in all populations. The rates of accumulation in the various populations were: (1, samples 4–34) 0.45% ± 0.07%, (3) 0.38% ± 0.05%, (5) 3.88% ± 0.5%, (6) 5.36% ± 0.5%, and (7) 1.04% ± 0.2%. The rate of accumulation in #3 agreed with previously determined spontaneous mutation rates for this chromosome (0.4%–0.5%) if one recalls that the two-week sample interval was probably somewhat shorter than the actual generation time in the populations. Even though the rate of accumulation of lethals in the chronically exposed populations need not represent the rate of mutation, it is interesting to note that exposure to 300 r per sample interval more than doubled the rate at which lethals accumulated and that the ratio between the rates of accumulation in populations 6 and 7 was approximately proportional to treatment. Lethals accumulated in populations 5 and 6 at quite similar rates—the difference in the calculated figures resulted primarily from the last sample of 5. It is impossible to tell at the present time whether this divergent sample from 5 represented genetic drift within a small population or the approach to equilibrium in a small population at a low frequency of lethals. Finally, it may be mentioned that the rates of accumulation in populations 1 and 3 were nearly identical following the initial elimination of lethals from population 1; the lethals eliminated quickly were probably those associated with translocations (Wallace, 1951).

Semilethals (table 3) are much rarer as a class than lethals; their accumulation in the control population and in population 7 was exceedingly slow (0.09 ± 0.015% and 0.05 ± 0.03%). Following the original x-radiation of population 1 semilethals were present and showed a subsequent increase

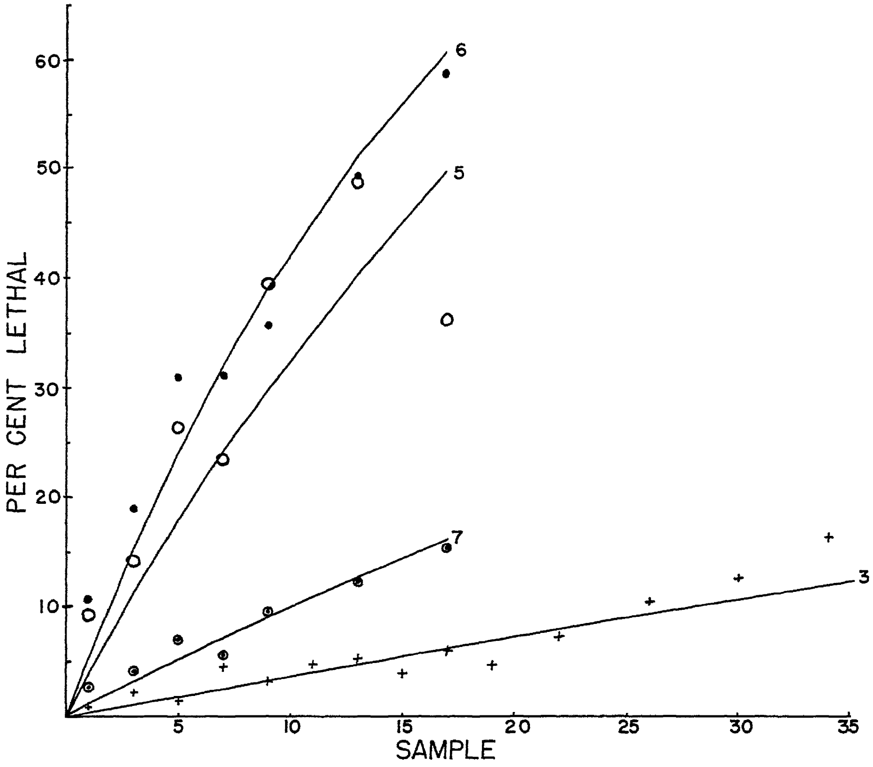

FIGURE 2. Frequencies of lethals recovered in samples of chromosomes taken from experimental populations; +, population 3; O, population 5; ●, population 6; ⊙, population 7. Theoretical lines fitted to the observed frequencies are identified by number.

TABLE 2

FREQUENCIES OF LETHALS IN SAMPLES OF CHROMOSOMES TAKEN FROM EXPERIMENTAL POPULATIONS OF *D. MELANOGASTER*

Sample*	#1	#3	#5	#6	#7
1	18.3%	0.8%	9.1%	10.7%	2.7%
2	14.1	0	...	...	...
3	11.6	2.2	14.2	19.0	4.2
4	10.1	...	...	...	...
5	...	1.4	26.4	31.0	7.0
6	10.9	...	...	...	...
7	...	4.6	23.6	31.3	5.8
8	13.7	...	...	...	...
9	...	3.2	39.4	35.7	9.6
10	14.6	...	...	...	...
11	...	4.9	...	...	...
12	14.5	...	...	...	...
13	...	5.2	48.9	49.2	12.2
14	13.6	...	...	...	...
15	...	4.0	...	...	...
16	18.8	...	...	...	...
17	...	6.1	36.2	58.8	15.5
18	18.7	...			
19	...	4.9			
20	13.8	...			
22	...	7.3			
24	20.0	...			
26	...	10.6			
28	25.4	...			
30	20.8	12.9			
34	24.2	16.5			

*Sample 1 of population 5 is really 20; of 6 and 7, 21.

exceeding that of population 3 (0.41 ± 0.07%; samples 1–20) and not differing significantly from those of populations 5 and 6 (0.51 ± 0.08%; 0.37 ± 0.07%).

Subvitals are genes with such small deleterious effects that they are difficult to demonstrate individually. Relevant data were obtained by computing the average frequency of wild type flies found in all non-lethal, non-semilethal test cultures of each sample. This average (table 4), obviously, would decrease if subvitals accumulate in the population. Unfortunately, since these data also reflect variations in culture conditions, only the data for populations 1 and 3 are sufficiently extensive to warrant discussion. The average viabilities of normal chromosomes from #1 were nearly always lower than those of population 3, their regressions (1. $Y = .3072 - .0002X$, $\sigma_b = .0002$; 3. $Y = .3172 - .0000X$, $\sigma_b = .0002$) have slopes not significantly different from 0. (During the time from sample 1 to sample 20 there was an apparently significant decrease in the average viabilities of normal chromosomes of population 1 ($p = .02$–$.05$) but over the whole course of the experiment there is no significant change.)

An estimation of the effects of radiation on populations can be obtained by selecting a character of presumed adaptive significance and making com-

TABLE 3

FREQUENCIES OF SEMILETHALS IN SAMPLES OF CHROMOSOMES TAKEN FROM EXPERIMENTAL POPULATIONS OF *D. MELANOGASTER*

Sample	#1	#3	#5	#6	#7
1	3.7	0	2.4	1.2	1.6
2	3.7	0	...	...	...
3	4.6	0	4.5	3.3	1.2
4	1.9	...	...	...	...
5	...	0	3.8	3.4	1.3
6	5.6	...	...	...	...
7	...	1.2	7.4	5.9	2.3
8	4.7	...	...	...	...
9	...	0.4	5.5	3.7	1.1
10	6.2	...	...	...	...
11	...	0.7	...	...	...
12	8.5	...	...	...	...
13	...	1.1	8.7	6.4	2.2
14	8.2	...	...	...	...
15	...	2.2	...	...	...
16	10.7	...	...	...	...
17	...	2.1	11.2	8.0	2.2
18	11.9	...			
19	...	1.5			
20	8.0	...			
22	...	1.9			
24	9.1	...			
26	...	1.5			
28	0	...			
30	6.2	1.7			
34	7.9	3.8			

parative studies of this trait in irradiated and non-irradiated populations. An ideal study would include all factors involved in the perpetuation of the population and would lead to a determination of the adaptive value obtaining under the experimental conditions. A complete study of this type is impractical for a number of reasons and so alternate methods must be used.

The method we chose for preliminary studies consisted of a modified series of routine test crosses. A typical individual in a population is the product of random mating and, consequently, carries chromosomes of different origins; the individual is heterozygous for many genes and, in all probability, its particular gene combination is unique. The method of reconstituting individuals representative of those found in a population is shown in fig. 3. At the time the F_2 flies were collected in the routine crosses, no information was available concerning the nature of the wild-type chromosome; this information was available only when the F_3 culture had been examined. In the modified test crosses (which may be referred to as "heterozygous crosses" in contradistinction to "homozygous crosses" of the standard test), F_2 flies from the test series were systematically outcrossed (males of a × females of b, males of b × females of c, males of c × females of d, ...males of n × females of a) as shown in fig. 2. Each

F_3 culture then contained *CyL*/+ flies of two types and wild flies heterozygous for two second chromosomes that originally were carried by different males. Depending upon the frequency of lethals and the probability of their allelism in the population, these heterozygous crosses occasionally lead to an F_3 culture containing no wild-type flies. Generally, however, the two chromosomes carried no lethals or semilethals in common and the frequency of wild flies was approximately the expected 33.3 per cent. The average frequency of wild-type flies in the whole array of combinations was taken as an estimate of the adaptive value of the population; obviously, it represented the relative viabilities of different types of larvae and pupae competing in slightly overcrowded culture conditions.

TABLE 4

AVERAGE FREQUENCIES OF WILD-TYPE FLIES IN TEST CULTURES OF NONLETHAL NON-SEMILETHAL CHROMOSOMES TAKEN FROM EXPERIMENTAL POPULATIONS OF D. MELANOGASTER

Sample	#1	#3	#5	#6	#7
1	30.66%	32.08%	31.75%	31.43%	31.52%
2	31.94	31.65	...	...	...
3	31.11	31.46	30.77	30.60	31.41
4	30.39	...	...	...	...
5	...	31.49	31.39	30.55	30.81
6	30.79	...	...	...	...
7	...	32.14	30.36	30.35	31.27
8	29.63	...	...	...	...
9	...	31.64	30.31	30.99	31.42
10	29.85	...	...	...	...
11	...	31.40	...	...	...
12	30.81	...	...	...	...
13	...	31.79	30.11	28.11	30.60
14	29.99	...	...	...	...
15	...	30.84	...	...	...
16	29.90	...	...	...	...
17	...	30.87	29.07	28.87	31.64
18	29.26	...			
19	...	32.25			
20	30.33	...			
22	...	32.77			
24	30.63	...			
26	...	32.04			
28	31.04	...			
30	30.83	32.77			
34	29.72	30.19			

The results of the heterozygous crosses are given in table 5. The interrelations of 3, 5, 6, and 7 were as one might expect; the chronic treatments resulted in lower estimated adaptive values and the effect varies with the treatment. Population 1, however, had distinctly more wild-type flies in the F_3 cultures than population 3. The relative adaptive values of the popu-

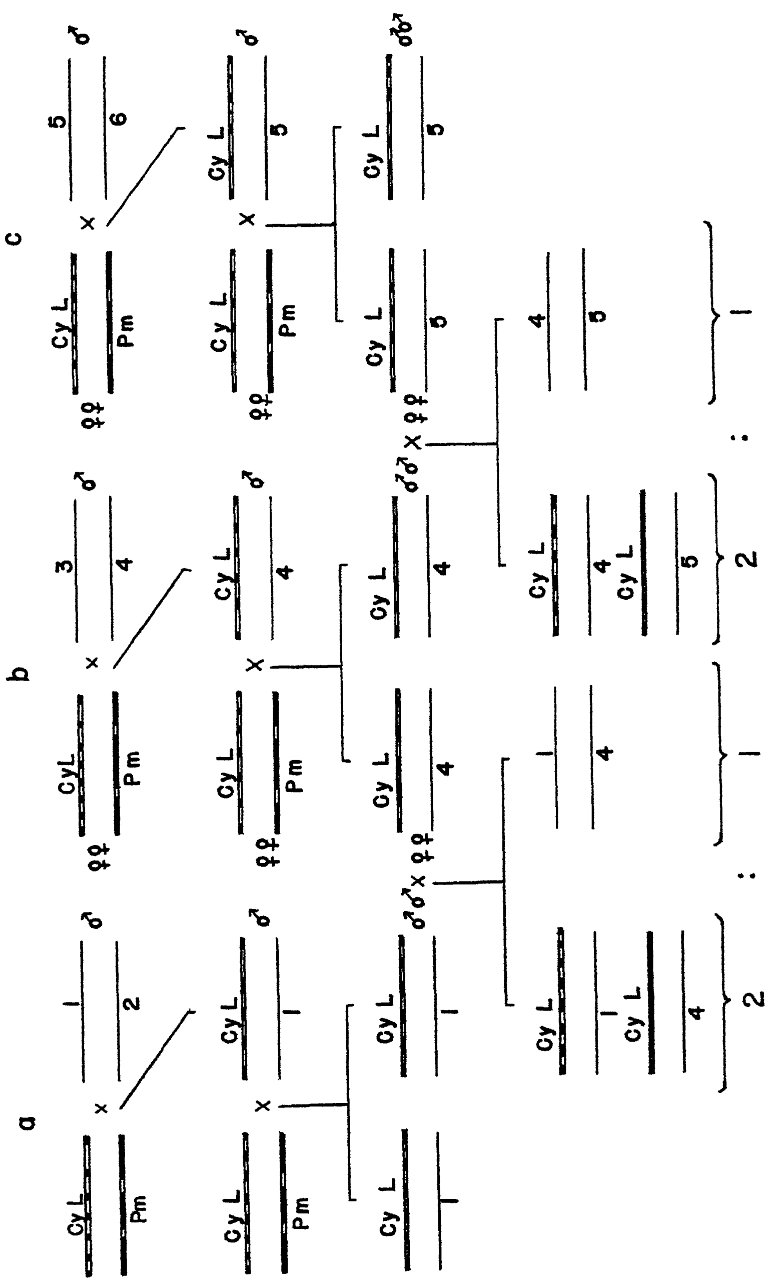

FIGURE 3. Mating system for studying viabilities of flies carrying second chromosomes of separate origin. Numbers and light lines represent wild-type chromosomes isolated from separate males.

TABLE 5

SUMMARY OF THE RESULTS OBTAINED FROM THE "HETEROZYGOUS" CROSSES DESCRIBED IN TEXT. (SEE FIG. 3)

Population	Sample	Number of combinations tested	% Lethal	% semi-lethal	Avg. freq. wild-type flies (%)	Estimated adaptive value of population
1	28	216	0	0	35.33	
1	32	312	0.3	0.3	34.19	
1	36	265	0	0.4	34.31	
1	40	201	0	0	36.90	
Total 1		994	0.1	0.2	35.02	1.04
3	32	274	0.4	0	33.12	
3	36	241	0	0	33.56	
3	40	207	0	0	34.82	
Total 3		722	0.1	0	33.75	1.00
5	30	163	0.6	0	31.48	
5	34	62	0	0	29.99	
5	38	165	0.6	0	31.06	
Total 5		390	0.5	0	31.07	.92
6	31	263	0.4	0	31.97	
6	35	209	0	0	31.74	
6	39	235	0.9	0	32.12	
Total 6		707	0.4	0	31.95	.95
7	31	256	0	0	32.91	
7	35	250	0	0.4	32.18	
7	39	224	0	0	33.48	
Total 7		730	0	0.1	32.83	.97

lations as revealed by this technique are: (1) 1.04 (3) 1.00 (5) .92 (6) .95 (7) .97.

DISCUSSION

The above account, while by no means exhaustive, describes and illustrates some of the more important types of data which can be extracted from experimental populations. Several questions concerning the evaluation and interpretation of the illustrative tables will now be discussed in more detail.

The first point that may be mentioned is the remarkable agreement between the rates of accumulation of lethals in populations 1 and 3 and the resultant constant difference of about 8 per cent in lethal frequency. This difference persisted for well over a year. The situation may be explained by assuming that the lethals which escaped elimination in population 1 during the first eight weeks (4 generations) were truly recessive and, because of the large number of lethal loci available (Wallace, 1950), were not appreciably eliminated by homozygosis. The situation can also be explained by assuming that the gradual elimination of radiation-induced lethals in population 1 was counterbalanced by a higher spontaneous rate of mutation of recessive lethals in that population. This explanation requires that the spontaneous rate of mutation to lethals changed in precise proportion to the change in the rate at which lethals were eliminated. In view of the long period over which the balance existed, it seems more likely that simple recessivity of lethals is the correct explanation. Proceeding from this premise, it is possible to compute the maximum degree of semidominance of lethals compatible with the data:

$$\frac{(b_3 + 1.3\,\sigma_{b_3}) - (b_1 - 1.3\,\sigma_{b_1})}{\text{average difference in lethal frequency}} = \frac{.0009}{.08} = 1\%$$

In the above expression, b_1 and b_3 are the slopes of the regressions of lethals in population 1 and 3, respectively; σ_{b_1} and σ_{b_3} are the errors of these slopes. The equation uses 1.3 σ in the ratio so that the confidence interval is nearer to the 95 per cent interval in general use; if 2 σ's are used, a 99.75 per cent interval is obtained and this is too large to permit a reasonably restrictive statement.

The data on average viabilities found in the different samples sheds light on the accumulation of subvital gene mutations in populations. If a population has initially only chromosomes which are normal when homozygous (average viability of 1), then the accumulation of subvitals of average selective disadvantage, $\bar{s}$, that arise by mutation at an average rate, $\bar{u}$, should lower the average viability by a factor $\overline{us}$ per generation. It may be postulated for convenience either that $\bar{u}$ is so low that most chromosomes carry only one subvital or that if a chromosome carries two subvitals, their combined effect when homozygous is additive.

The interesting populations in connection with subvitals are 1 and 3 because of their greater age and more numerous samples. The slope, b, of the regression is a function of $\overline{us}$; if there is no appreciable elimination of subvitals from the populations, $3b = \overline{us}$ (b is calculated in relation to an expected frequency of wild-type flies of approximately 33.3 per cent while $\overline{us}$ is given in relation to an average viability of 1). It is obvious that the product of $\bar{u}$ and $\bar{s}$ is small; in neither population 1 nor 3 has the slope differed significantly from O. Because of the experimental variations, values of $\overline{us}$ equal to .0012 or .0018 would not be inconsistent with the data. The constant difference between the average viabilities of the two populations (as reflected by the similarity of the slopes of the regressions) argues against any considerable elimination of subvitals in the heterozygous condition. The data are not sufficiently precise to allow a decision regarding the initial increase in the average viability in population 1; the increase may be the result of sampling error or it may reflect the elimination of subvitals associated with translocations (these subvitals would be effectively semilethal in the heterozygous condition).

The most important characteristic of an irradiated population is its adaptive value. The purpose in studying rates of mutation to various types of deleterious alleles at separate loci is that an estimate of the adaptive value can be made from a knowledge of the frequencies of homozygosity and the degree of disability of each deleterious homozygote. The result of such a study would be the comparison of an ideal adaptive value of 1 with calculated adaptive values of $1-s_1$, $1-s_2$, etc., where s is the decrease expected from the mutant genes.

The data obtained from the heterozygous crosses throw doubt on this simple procedure. For the sake of simplicity, consider populations 1, 3, and 7. The chromosomal data show that, at the time of the heterozygous crosses, population one had more lethals, more semilethals, and a lower average viability of flies homozygous for "normal" chromosomes than either 3 or 7 and that the two latter populations were quite similar in these respects. The occurrence of lethal and semilethal combinations in the heterozygous crosses indicated that the chance of two chromosomes of different origin having deleterious genes at the same locus was approximately the same in all three populations. However, when all tested combinations are considered, population 1 produced a higher frequency of wild-type flies than either 3 or 7 and 7 produced fewer than 3. The adaptive value, therefore, seems to be at least partially independent of observed frequencies of "deleterious" genes in the population.

The conclusion reached above depends, of course, on the technique of estimating the adaptive value. The most serious flaw in the method would arise if dominant subvitals were to distort the observed ratios of *CyL*/+: +/+ flies by decreasing the frequency of *CyL*/+. We have noted, however, that the average viabilities of normal chromosomes in 1 and 3 did not converge; this indicates that subvitals in 1 were not semidominant. In the case of population 7 where genes were constantly mutating it was possible that some dominant subvitals were present, but this merely makes the calculated adaptive value of this population too high; in our argument we are regarding it as lower than that of population 3.

The results of the experiment have indicated, in brief, that while an examination of the individual chromosomes of a population may reveal that these are generally "deleterious" when homozygous, an examination of *pairs* of unrelated chromosomes from the same population may reveal that these pairs are distinctly superior. An insight into population dynamics is gained from this seeming contradiction. In a population (of Drosophila, for instance) where deaths are numerous between the fertilization of the eggs and attainment of sexual maturity, selection acts on combinations of genes; individuals survive that are carrying adaptively superior combinations. Meiosis and fertilization break up the selected combinations of each generation and make new ones from the available genes. The process is repeated again and again and the degree of "co-adaptation" (Dobzhansky, 1950) increases. The difference in the adaptive values of populations 1 and 3 can be explained on the basis of a larger number of potential combinations available in 1 than in 3. The currently higher adaptive value of population

1 could exist not merely *in spite of* but *because of* the original treatment. This may be a temporary affair; as more genetic diversity develops in 3, the adaptive values of the two populations will probably converge.

The low adaptive value of population 7 can be explained on the same basis but with one important facet remaining unknown. The basic selective process for superior combinations ("superior" in the population cage environment) could proceed in this population as in the others with a reshuffling of chromosomes occurring during meiosis and fertilization. The chromosomes that are reshuffled, however, are undergoing chronic irradiation and because of the mutations constantly occurring, are not the chromosomes that were originally chosen. This in itself is not a novel situation; spontaneous mutation leads to a similar condition in all populations. The precise reason why the adaptive value of 7 is lower than that of 1 or 3 is the important point: Has the number of generations that the population has existed been too few to allow for a comparable selection of heterosis? Does the amount of irradiation the population receives slow up the selective process substantially? Is selection inadequate to maintain superior combinations under these conditions? Time and additional experimentation will give the answers to some of these questions. The lower frequency of wild-type flies in populations 5 and 6 than in 7 indicate that chronic irradiation might disrupt coadaptation to a considerable degree. The data now available are not sufficient to allow the calculation of regressions of adaptive values. Other estimates of adaptive values are being obtained so that the data will not be limited solely to larval competition in F_3 culture bottles.

It must be re-emphasized in conclusion that the purpose of this paper was to introduce an experimental method of attacking a particular problem by presenting and discussing some typical data. The most important conclusions emerging at present are that heterotic combinations are selected for and become established in populations at an extremely rapid rate and that radiation-induced genetic variants are seemingly incorporated into these combinations. A corollary of practical importance is that, in estimating the genetic effects of radiations on populations, consideration must be given to the existence of coadapted genetic systems.

SUMMARY

A general account is given of studies made on experimental populations of *Drosophila melanogaster* which are exposed to radiations. A consideration of the frequencies of lethals, semilethals, and subvitals within the populations and of the estimates of the adaptive values of the populations suggests that heterotic gene combinations have been developed within some of these populations. The bearing these results have on estimation of radiation damage resulting from gene mutation is briefly mentioned.

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DISCRIMINATION OF STREAM ODORS BY FISHES AND ITS RELATION TO PARENT STREAM BEHAVIOR[1]

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INTRODUCTION

The role of sensory mechanisms in orientation of fishes, especially in relation to homing, remains a mystery notwithstanding many efforts to solve this problem. It is the object of this paper to point up again the possibility that the sense of smell may play an important part in directing fishes to their home waters. The primitive character of this sensory system, its evolutionary constancy, its extreme sensitivity in comparison to other receptor processes, and its capacity to serve as a memory-evoking mechanism, all point to a working hypothesis—that olfactory stimuli may be factors in the homing of migrating fishes.

Scheer's (1939) review has summarized the results of the homing instinct in salmon, which he defines: "... salmon or trout hatched, and reared in a particular region will, upon returning to fresh water, return in the great majority of cases to the same region, even from considerable distances." While Scheer cited several workers whose marked fish had strayed from the home stream, one cannot fail to be impressed with the convincing data of Pritchard, Foerster and Clemens (1939) illustrating the accuracy of homing. Of 469,326 specimens of *Oncorhyncus nerka* marked before the oceanward migration, almost 11,000 returned to their parent stream, the Fraser River tributary of Cultus Lake. There was no straying. Allowing for heavy mortality at sea, these results of precise homing behavior defy explanation and point up the necessity for assuming a sense of great acuity.

While the idea has been mentioned by some writers (Scheuring, 1930, and Kyle, 1926) that homing in fishes may be ascribed to scent-perceiving tissues, details of such sensory control of migration have not been made clear. Craigie (1926) released 500 sockeye salmon, in half of which the olfactory nerves had been severed. Judging from later recaptures of the tagged normal and operated fishes, it was evident that the migratory behavior of the latter had been somewhat interfered with. White (1934) suggested that the fish oriented to milt shed into the water by precocious males; he presented no evidence on how it was detected.

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In the above observations, it could not be clearly proved that fishes orient on the basis of olfaction; nor has the idea been proposed that the home water carries a distinctive odor which through discrimination and memory may serve as a directing guide to the fishes in orienting toward and reaching its original habitat. The chief objective of our experiments was to determine whether streams do have characteristic odors and if fishes can discriminate between them, and whether they can retain in memory habits of orientation learned with respect to these odors.

It may be desirable, at this point, to define clearly the requirements of an odor which is to serve as a "sign-post" for returning salmon. First of all, it must remain relatively constant in any one stream over a period of years because an interval of three to five years may elapse between original learning of the olfactory controlled reaction and its reinstatement, upon return to the vicinity of the home water to spawn.

Since it is known that some salmon return to their home streams at the early age of three years, while others delay until four or five, it can easily be seen that the odor cannot be cyclical in nature, but must be present in the same form year in and year out. In this instance then, changes in the odor must not take place with more rapidity than evolution of the species, if the homing of the salmon is not to be disrupted.

The second condition which must be placed upon the odor is that it must have significance only to those returning migrants which had been conditioned to it during their freshwater sojourn; while being neutral to all others for it would seem that any odor, or substance, which was an attractant or repellent would induce salmon to enter a stream or tributary irrespective of whether or not they were native to the stream.

There is yet another restriction which must be placed upon a homing odor. It must remain detectable even though the stream be changed severely in chemical and physical characteristics, for salmon will continue to attempt to return to a stream even though that stream may have been seriously polluted, or gutted by floods, during the time the salmon were at sea.

It should be stated that the authors are not precluding the possibility that there may be other factors contributing to the ability of the salmon to return to their home waters. However, it is our opinion that of all the theories which have been suggested, that of a conditioned olfactory response is indeed plausible and seems to merit further exploration.

In this laboratory we (1949) demonstrated that natural odors, such as dilute rinses of aquatic plants, were perceived and discriminated by the bluntnose minnow (*Hyborhynchus notatus* Raf.). Moreover, these minnows were trained to respond to concentrations of phenol far below the odor threshold for man (Hasler and Wisby, 1950). Therefore, because our laboratory experience has been more extensive with the bluntnose minnow, it was adopted as the assay animal. Subsequently salmon were tested also.

METHODS AND MATERIALS

The water for this series of training tests was obtained from two creeks which drained watersheds of different edaphic conditions. One sample was taken from Otter Creek at Meyer's Hill (Baxter's Hollow) where the creek crosses the line between sections 32 and 33 (T.11N., R.6E.). The other was procured from the north branch of Honey Creek in the S. E. corner of section 11 (T.10N., R.4E.) of the same quadrangle.

Otter Creek heads in an area composed of about 90 per cent quartzite rock, the remainder being mainly sandstone and dolomite. Honey Creek, on the other hand, runs over moraines composed principally of sandstone (95 per cent) cemented with dolomite, with lesser amounts of quartzite (Wanenmacher, Twenhofel, and Raasch, 1934).

Water from both creeks was collected in five-gallon jugs. That which was used for the first series of tests was packaged in polyethelene bags, which were then sealed with an electric curling iron. Waxed cardboard containers were substituted for the polyethelene bags in later tests. The filled containers were placed in a deep freezer and kept until used. Before a test was run, the ice was melted and allowed to come to room temperature.

It was the immediate intent of this experiment to determine if it were possible to obtain an olfactory discrimination between Otter Creek and Honey Creek by the bluntnose minnow. The minnows were trained in such a manner that they learned to associate the odor of one of the streams with food and the odor of the other with punishment.

The equipment used by the authors (1949 and 1950) was suitable for conducting this series of training tests. It consisted of several seven gallon aquaria, each with a siphon-airlift circulation system installed in both ends (fig. 1). Water was siphoned from the aquarium, returned by air pressure, and discharged into a 6-inch funnel which was suspended above the tank. The funnel was connected to a glass tube which lay across the end of the aquarium. Perforations in the tube directed the incoming water across the bottom of the aquarium. Water from the jet on one side flowed only about halfway across, because there it met the stream from the other end, and both were deflected upward. This produced two currents or convection cells, each of which involved one half of the tank. Water samples containing the odors were introduced into the aquarium by means of a separatory funnel, which was connected to the siphon tube after it left the tank.

An objection to the two-electrode punishment system, as described by the authors (*l.c.*), was overcome by introducing a third electrode. Formerly, it was difficult to punish a fish which entered the end zone more than two to four centimeters above the electrodes. Also, a fish between the electrodes was apt to be injured, as a shock was administered. With a third electrode, located about two inches above the one in the corner, it was possible to punish, without injuring, any fish entering the end zone below the level of the new electrode. Thus, a region (2″ x 2″ x 6″) bounded on the bottom by the two electrodes on the floor of the aquarium was designated as the "end zone," that is, the place where the fish were fed or punished by electric shock (2.3 volts; 20 milliamperes) depending on which odor was being introduced. Also, higher voltages could be used without adversely affecting the fish, thereby impressing the training to the negative odor.

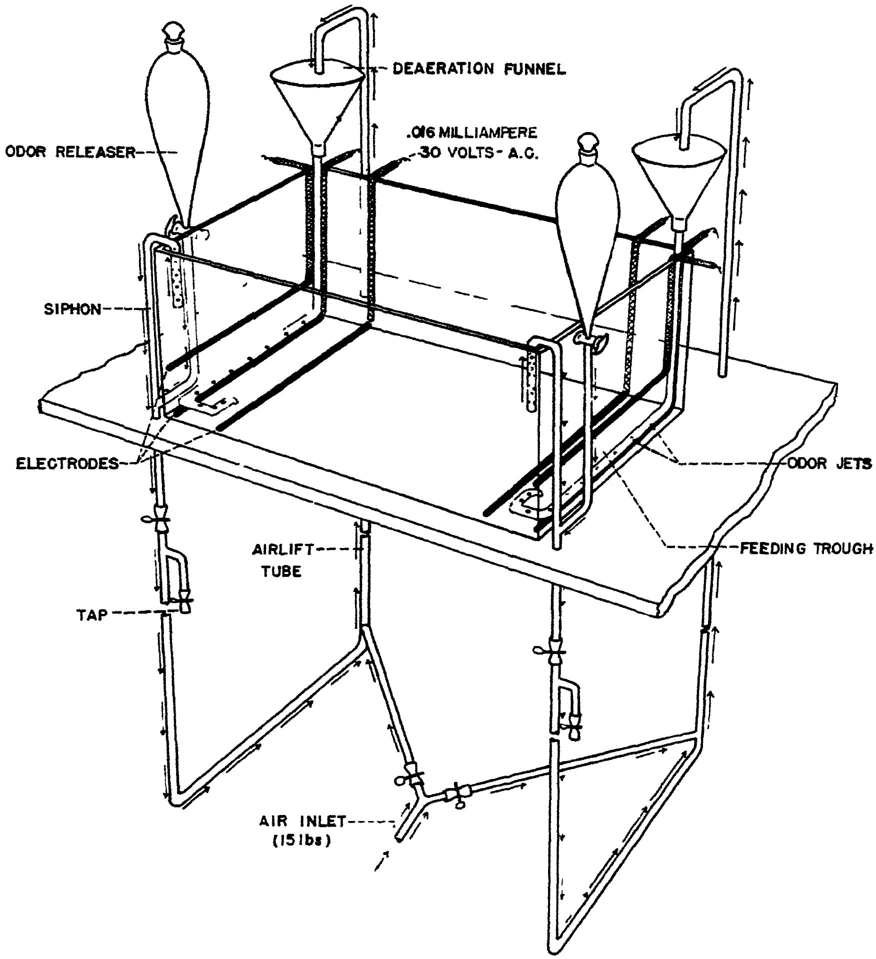

FIGURE 1. Experimental tank with siphon-airlift circulation system.

The fish were rewarded by introducing food, pressed on perforated celluloid strips, into the end zone. Since, in this method of training, hunger is the principal motivating force, tests were timed at those intervals when hunger motivation was at its greatest. An attempt was made to test at different times each day, and to feed no more than was necessary for the well-being of the experimental animals. The fish were fed very heavily every sixth day, and tests omitted on the seventh, because a mere subsistence diet such as this would, in time, have harmed them.

With a set of electrodes and an odor outlet in each end of the aquarium, it was possible to randomize the presentation of odors. A table of random numbers (Snedecor, 1948) was used to determine for each day which odor was to be presented first, and from which end of the aquarium. In order to eliminate cueing to the operator, the fish were blinded by chemical cautery (injection of phemerol into the posterior chamber of the eye).

To eliminate any extraneous stimuli that the fish might receive during training, the following additional precautions were taken:

1. The aquaria were insulated from gross mechanical shocks by mounting them on sponge rubber.

2. Introduction of the odor was accomplished in such a manner that it did not alter the dimensions of the stream of water which was constantly flowing into the aquarium.

3. The observer whispered scores to a tape recorder over a telephone system to avoid any association with changes in pitch or rapidity of the observer's speech.

Fishes of two aquaria received positive training to water of Otter Creek, that is, fish were fed immediately after the test, and negative training to water of Honey Creek, that is, fish were punished by a light electrical current if they entered the end zone during the time this water was being introduced. Two other aquaria were trained to the same two odors, but with opposite meanings; Honey Creek was the positive odor; Otter Creek, the negative. If training were accomplished the fish should eventually associate food with the positive scent, and therefore enter the end zone. The negative scent would be associated with punishment and the fish should stay out of the end zone.

It should be pointed up that the fish were fatigued to the natural odors of the aquarium so that introduction of an unfamiliar water was immediately detected if in a perceptible concentration. In order to assure a forceful stimulus the creek water was diluted only by one-half when it was put into the separatory funnel. A minimal detectable concentration was not established.

The scoring procedure was essentially the same as that previously reported with but few modifications. A new method of recording the scores, using I.B.M. cards, was devised and will be described in a future paper.

Each fish was given a distinctive mark by attaching a bead to its back with a tantalum wire suture. Since each bead was of a unique color it was an easy matter to record the reaction of the individual fish.

The score for the entire aquarium was obtained from the mean scores of the six fish composing the group. Scores for three different measurements of reaction were obtained:

1. *Test:* Scores recorded during a sixty second test period coinciding with exposure to the odor. The test period was divided into intervals of 5 seconds. A fish scored once if it entered the end zone in a 5-second interval. Additional entries by the same fish in this same period were not counted. Thus, one fish could accumulate a total of twelve points per test. Arbitrarily these points were expressed as sixty seconds, that is, five seconds allotted to each point.

2. *Hesitation:* Scores for those five-second intervals intervening between the time the fish perceived the odor until it entered the end zone. Percep-

tion of the odor is manifested by a sudden change in behavior of the fish. It is especially obvious in trained fish and is easily recognized by the observer after short familiarity with the fish.

3. *Pretest:* Scores of random movement during a sixty second period prior to introduction of the odor.

REACTION TO TRAINING ODORS

Positive and negative tests were given daily and the fishes' scores recorded.

At the outset the fishes showed natural unlearned orientative responses for the odor of either creek, that is, they entered the end zone more often during the introduction of the odor on either the negative or the positive test than during the pretest. Not until later in the training sequence did they discriminate between them.

Tables 1a and 1b give records of activity according to the three items of behavior listed above. It is evident that the minnows learned equally well irrespective of whether Otter Creek (draining quartzite watershed) was positive (table 1b) or negative (table 1a). A noticeable degree of discrimination was accomplished in a month of training. Data presented in the tables are averages for training at the end of each month.

After reaching the discrimination level shown in tables 1a and 1b training was continued for two more months in order to attain the maximum level of discrimination, or the plateau in the learning curve.

RETENTION OF LEARNING

If salmon, also, are able to detect characteristic stream odors, and if these odors are to serve as orienting influences on migrating fishes, the early associations to the odor must be retained during the four-year sojourn at sea.

An attempt was therefore made to determine the length of retention of discrimination in the bluntnose minnow. Daily training was stopped and odors were presented weekly, without reward or punishment. After six weeks the fish were confusing the two odors so completely it was apparent that discrimination no longer existed (table 2a).

That this method of testing does not produce a clear-cut measure of actual retention is well known to psychologists. The procedure followed involves detraining through repeated testing, and through interference habits set up by routine daily feeding between tests. That is, an animal which has been trained to associate food with an odor, will be subjected to the reverse of this training process if fed without prior introduction of the odor. Thus, the minnows, during these tests, were actually being detrained, and the results of the tests can only be considered to be an absolute minimum indication of true retention.

It should be kept in mind that the life span of the bluntnose minnow is only two years. Hence, training was started at the senile stage, whereas salmon would be conditioned to the stream odor while young.

TABLE 1a

MEAN TRAINING SCORES IN SECONDS FOR FISHES REACTING TO ODOR STIMULI WHERE THE POSITIVE ODOR[¶] WAS WATER FROM OTTER CREEK, AND THE NEGATIVE ODOR[†] WAS WATER FROM HONEY CREEK

Tank No.:		7			8		
Month of training[*]	Odor tested	Pretest[‡]	Test[§]	Hesitation[‖]	Pretest	Test	Hesitation
First	positive	63	97	69	39	142	43
	negative	66	46	104	35	61	82
Second	positive	85	181	55	57	160	47
	negative	73	20	200	52	18	196
Third	positive	68	194	46	43	176	31
	negative	47	21	217	40	19	209
Sixth	positive	47	200	6	39	242	9
	negative	39	15	241	40	13	226

TABLE 1b

POSITIVE ODOR: HONEY CREEK; NEGATIVE ODOR: OTTER CREEK

Tank No.:		2			3		
Month of training[*]	Odor tested	Pretest	Test	Hesitation	Pretest	Test	Hesitation
First	positive	25	76	76	40	113	34
	negative	58	52	132	68	43	146
Second	positive	47	123	56	87	156	35
	negative	42	40	180	76	44	137
Third	positive	43	146	47	64	182	23
	negative	57	27	203	39	19	129
Sixth	positive	46	343	6	34	336	0
	negative	31	13	285	40	17	273

[*] Records for tests made at this stage of training.

[¶] Positive odor: The odor with which fish had been trained to associate reward.

[†] Negative odor: The odor with which fish had been trained to associate punishment. No punishment accompanied these tests.

[‡] Pretest: Scores from random movement during 60 seconds preceding test.

[§] Test: Scores during 60 second period coinciding with exposure to odor.

[‖] Hesitation: Scores for 5 second intervals from time they perceive the odor until they enter the scoring zone.

Early conditioning in lower animals is known to influence their adult habits. Thorpe (1938) concluded that the ichneumon fly, Nemertis, has an inherited oviposition response to larvae of the flour moth, Ephestia, but not to larvae of the bee-wax moth, Meliphora. However it responds to the latter if raised on it during the larval feeding period, and given equal opportunity to oviposit on either species. And, a case closer to the point, Fish and Hanavan (1948) furnish good evidence that runs of salmon were established in renovated streams from migrants unable to pass the newly

TABLE 2a

EXTINCTION TESTS: SCORES IN SECONDS FOR OLD FISHES REACTING TO ODOR STIMULI OF THE TRAINING ODORS

Tank No.:		2			3			8		
Days after training*	Water tested	Pretest	Test	Hesitation	Pretest	Test	Hesitation	Pretest	Test	Hesitation
7	Honey Creek†	50	320	0	50	325	10	40	15	150
	Otter Creek	20	40	220	50	15	220	35	235	0
17	Honey Creek	60	190	0	45	325	10	65	70	180
	Otter Creek	60	50	125	70	60	115	80	270	0
29	Honey Creek	50	150	25	60	175	60	70	95	125
	Otter Creek	70	145	60	40	100	85	65	90	90
39	Honey Creek	110	115	80	45	75	75	115	125	130
	Otter Creek	120	130	120	25	75	130	120	140	115

*Records for tests made on this day after training ceased.

†In tanks 2 and 3, Honey Creek was the positive odor; Otter Creek, negative. In tank 8, Otter Creek was the positive odor; Honey Creek, negative.

built Grand Coulee dam. It appears here that eggs from these relocated fish yielded survivors which responded to some characteristic of an adopted stream to which they had been conditioned.

It was then felt that it would be desirable to know whether or not the retention of learning in young fishes is longer than in old, which should be the case if this hypothesis is to be applied to salmon. For this reason, an entirely new set of yearling minnows was trained to the level of discrimination previously attained with the same creek waters and methods described above. It was found that these fish were able to discriminate between the odors for fifteen weeks after cessation of training (table 2b).

TABLE 2b

EXTINCTION TESTS: SCORES IN SECONDS FOR YOUNG FISHES REACTING TO ODOR STIMULI OF THE TRAINING ODORS

Tank No.:		2		5	
Days after training	Water tested	Test	Hesitation	Test	Hesitation
0	Otter Creek*	322	8	22	297
	Honey Creek†	21	304	315	17
52	Otter Creek	276	15	102	254
	Honey Creek	53	272	304	24
66	Otter Creek	261	34	43	266
	Honey Creek	29	256	205	51
95	Otter Creek	93	97	102	119
	Honey Creek	71	134	182	96
105	Otter Creek	74	114	107	76
	Honey Creek	82	127	121	90

*Otter Creek was the positive odor in tank 2, and the negative odor in tank 5.
†Honey Creek was the positive odor in tank 5, and the negative odor in tank 2.

There is a record of true retention of learning in fishes, where detraining was not involved. Stetter (1929), in a study of sound discrimination, reported that the minnow *Phoxinus laevis* responded to tones 229 days after it had been conditioned by training.

SEASONAL INFLUENCE ON CHARACTER OF ODOR

It was postulated, in the introduction, that a stream must retain its characteristic odor throughout the year, as well as over a period of years, in order to be of value in explaining parent stream behavior. To test the possibility of seasonal changes in odor, samples were collected during winter and presented to fish that had been trained to water from the same streams which had been collected in summer. The fish responded equally well to this water, indicating that the odor characteristics recognized by the fish in these two streams did not lose their identity with the change in season.

EVIDENCE OF OLFACTORY DETECTION OF STREAM ODORS

To determine if these differences in water are perceived by tissues of the fish's nose, the olfactory capsules of trained fishes were destroyed by heat cautery. After the wound had healed, these fish were again tested with the training odors. There was no response; nor did they participate in the reaction when placed in an aquarium with normal, trained minnows. It can thus be seen that a reaction to the substance is dependent on the olfactory system and on individual perception of the odor, and is not a "follow the leader" phenomenon.

That the latter is true can also be shown by placing a blinded, but otherwise normal, fish, which has received positive training to one odor and negative training to the other, in an aquarium with fishes which have received the reverse training. When one of the training odors is then introduced into the aquarium, the odd fish exhibits a response which is the opposite of that being demonstrated by the resident fishes.

NATURE OF THE ODOR

With this proof that the olfactory receptors were stimulated by a property of the creek water, it was logical to wonder what the substance was. A standard chemical analysis was made of samples of the water which had been frozen (table 3). While chemical differences existed, it must be remembered that no fishes have ever been trained to differentiate, by smell, various levels of nitrogen, phosphorus, alkalinity, or pH. Of significance in these analyses is the zero CO_2, because Powers (1939) postulated that salmon might be able to follow gradients of CO_2 in streams or of water masses. If the salmon responds as does the minnow, this factor can be ruled out as a signal to a migratory route.

Differences in total organic nitrogen of the two streams were quite marked. Since most odorous compounds are organic in nature, it seems quite likely that the elements detected by the minnows may be located in this fraction.

INTERPRETATION OF GENERALIZATION TEST DATA

Fish trained to a positive and negative odor may be tested against a new third odor. This technique is known as a generalization test, commonly used in psychological experiments. Here it is presumed that the degree of similarity between a new stimulus and either of the stimuli to which the fish have been conditioned, is determined by observing how nearly the former evokes the behavior associated with either of the learned stimuli.

In these experiments, as mentioned previously, fishes of two aquaria received positive training to water of Honey Creek, and negative training to water of Otter Creek, while two other aquaria were trained to the same two odors but with opposite meanings attached. Thus a third (generalization) odor which produces a negative response in fishes of one aquarium, should

TABLE 3

CHEMICAL COMPOSITION OF THE TEST WATER, EXPRESSED IN P.P.M.

	Otter Creek	Honey Creek
NH_3-N	0.04	...
NO_2-N	0.23	0.59
NO_3-N	0.16	0.50
T.O.N.	0.23	0.59
Sol. P.	0.02	0.15
pH	8.2	8.5
Alkalinity $CaCO_3$	19.0	25.0
Free CO_2	0.0	0.0

produce a positive reaction in the fishes of the opposite aquarium. This, however, is not always the case (authors, *l.c.*). An odor which evokes a negative response in an aquarium in which Otter Creek is negative, may not produce a positive reaction in the aquarium in which Otter Creek is positive. On the other hand, a new odor which is accorded a positive reaction in one aquarium, will always receive negative treatment in the aquarium which has received the opposite training. This would imply that avoidance of electrical shock, which in training is associated with the negative odor, is a stronger motivating factor than hunger. A negative reaction to a generalization odor in both aquaria of an oppositely trained pair, may be the result of an avoidance response to a strange odor. A new (generalization) odor can only be considered similar to a given training odor when it produces the same reaction as that training odor in both aquaria of an oppositely trained pair.

RESPONSE TO ASH RESIDUE

The first generalization test was designed to determine whether the odor to which the fishes responded was organic or inorganic in nature (table 4)[2]. The ash residue of each stream was dissolved in distilled water, diluted to the original volume, placed in containers, frozen and tested with the trained minnows precisely as before. A complete set of experiments proved that the fish did not associate the residue with either of their training odors since their scores were within the range of the control tests on distilled water. This established more clearly that the odorous stimuli might be either in the organic fraction or an organic-inorganic complex.

RESPONSES TO DISTILLATES

(a) To fraction volatile at 100°C. and its residue.

Water from both creeks was distilled at 100°C. at atmospheric pressure and tests were conducted with both the distillate and the rediluted residue.

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[2] With reference to tests on organic fractions, table 4 lists results only of two aquaria of a duplicate set. To save space, duplicates were not included since they did not add to the significance of the results.

TABLE 4

MEAN TRAINING SCORES IN SECONDS FOR FISHES REACTING TO ODORS OF OTTER CREEK AND HONEY CREEK AND TO VARIOUS FRACTIONS OF THESE WATERS.*

Tank No.:	1			4		
Odor tested	Pretest	Test	Hesitation	Pretest	Test	Hesitation
Otter Creek	45	326	5	40	22	291
Honey Creek	48	18	298	46	313	14
Inorganic fraction of Otter Creek	....	45	145	....	88	158
Inorganic fraction of Honey Creek	....	55	178	....	75	112
Distillate of Otter Creek (100°C)	....	56	176	....	49	164
Distillate of Honey Creek (100°C)	....	50	182	....	48	159
Residue of Otter Creek (100°C)	....	61	190	....	66	177
Residue of Honey Creek (100°C)	....	58	183	....	64	174
Distillate of Otter Creek (25°C)	....	276	36	....	27	251
Distillate of Honey Creek (25°C)	....	24	247	....	292	30
Residue of Otter Creek (25°C)	....	149	78	....	56	217
Residue of Honey Creek (25°C)	....	32	200	....	184	94
Residue and distillate of Otter Creek (25°C)	....	297	21	....	36	247
Residue and distillate of Honey Creek (25°C)	....	20	276	....	304	21
Distilled water control	....	40	155	....	75	160

*Otter Creek was the positive odor in aquarium No. 1, and the negative odor in aquarium No. 4.

Honey Creek was the negative odor in aquarium No. 1, and the positive odor in aquarium No. 4.

The fish proved to be as oblivious of these odors as of the above (table 4); the chemical stimulant apparently being destroyed by heat. Control tests with pyrex distilled water produced scores of the same order of magnitude indicating that any reaction produced was due to normal handling of the frozen water. It should perhaps be mentioned that in the generalization tests described so far, difficulty was experienced in trying to determine whether the scores were the result of random movement of the fishes or of responses to the solution introduced. The characteristic excitatory reaction, which was described as accompanying detection of one of the training odors, was completely lacking.

(b) To fraction volatile at 25°C. and its residue.

The next series of tests was conducted with the residue and distillate of water that had been vacuum distilled (pressure: approximately 22 mm.

The distillate and its residue were first mixed and tested to determine if the process of distillation had somehow altered the nature of the odor. It was noted by the observer that the intensity of the reaction to the positive odor was diminished. However, it was not possible to demonstrate a significant difference ($X^2 = 1.46$, $p = 0.49$) between these scores and those of the normal positive odor.

The fishes were then subjected to each of the two fractions separately. As can be seen from table 4, the scores of the reaction to the distillate more nearly resembled the scores of the response to the original water than did those of the redissolved residue. In fact, there was no significant difference between positive scores for the distillate and for the original water of either creek ($X^2 = 2.23$, $p = 0.4$), but a highly significant one between scores for the rediluted residue and the original water ($X^2 = 66.95$, $p = 0.0001$). These comparisons were made only on positive test scores since, as was mentioned previously, factors other than a similarity between odors may produce a negative response.

This biological assay, then, indicated that the odorous stimulant was contained in the volatile fraction and might thus be presumed to be an aromatic substance.

SIGNIFICANCE AND APPLICATION

Most of the evidence for the reliability of parent stream behavior in fishes is found in the literature on the salmon. When it was decided to initiate a series of experiments to attempt to discover the mechanism behind parent stream behavior, therefore, they were designed to lead eventually to a series of actual tests in the field with salmon.

In this research, one major barrier to the hypothesis that fish orient to their home streams, has been explored. That is, it was shown that some streams, at least, have odor characteristics which can serve to produce persisting differential responses in certain fishes. Furthermore, the results of generalization tests indicate strongly that the odors of streams are aromatic substances present in the volatile organic fraction. However, our evidence for olfactory discrimination of stream water by fishes does not constitute proof that parent stream behavior is not also controlled by other factors.

Scheer (1939) in his review of the homing instinct writes:

> The existence of a certain ability to follow a definite migratory course leads naturally to the question, what sense or senses are involved?... Ward (1921a, b, 1939a, b) studied *O. nerka* in the Copper River, Alaska, and concluded that when presented with a choice between two tributaries, the salmon invariably chooses the one with the cooler water.... Powers (1939) has attributed the direction taken by *O. nerka* to (a) gradients of salinity in the sea, and (b) gradients of CO_2 tension in the sea and in rivers. Although the suggestions made by these writers are of some value in indicating possibilities, neither has taken into consideration the fact that a run of fishes, whether in the sea or in a river, may divide, some passing into one river or tributary while others continue in their previous course. Neither author is willing to agree with the parent stream theory as stated above.

After citing Craigie's inconclusive results (see Introduction) Scheer continues:

> We must then say that at present we know little or nothing about any mechanism which might enable the salmon to find his way to a particular stream or tributary. Indeed we may say as Sumner (1939) has, that it is difficult to see how any known sense or combination of senses would be adequate, in view of the fact that variations in a certain property of a given stream might be greater from year to year than would be the differences between the stream and a nearby one. Studies directed at this feature of the "homing" question should prove of the greatest of interest.

In our studies we believe to have presented evidence which answers Sumner's objections. Also the mere fact that it can be demonstrated that fishes are attracted or repelled by substances such as CO_2 (Powers, 1943) does not signify that the salmon are responding to it in homing. Indeed, it would seem to preclude that possibility. If this were the case salmon might be expected to follow a gradient regardless of their origin.

One of the characteristics which a stream must have to fulfill the conditions of our thesis is that the substance, to which the fishes are responding on their return journey, must remain detectable even though the stream be changed severely in chemical and physical characteristics. Salmon continue to return regardless of pollution, floods, and changes in weather. These things do alter the materials in the stream, but on the basis of our evidence it appears that aromatics derived from the vegetation and soils of the watershed lend a distinctive odor which can be perceived, learned and recognized again after a protracted period of non-exposure. The aromatic characteristic of a watershed, filtering into the stream, might be surmised to remain constant over long periods.

Additional collateral indications of the importance of the sense of smell in the life of a fish comes from a large series of studies by von Frisch (1941) and his students. Their results attest the extreme sensitivity of the fish nose to natural substances, for they show that fish have been found capable of recognizing one another by scent, and that they may be alarmed at extremely dilute emanations (Schreckstoff) from injured fish skin. It would seem too that the acuity of the sense of smell in fishes is of similar sensitivity as that of dogs and insects where but a few molecules stimulate the end organ. In contrast, the common chemical sense (Powers, 1943) and the ability to discriminate temperature differences (Dijgraff, 1940) are crude senses when compared with the olfactory system.

Techniques have been developed whereby it is now possible to hatch and maintain salmon through the fingerling stage in aquaria at Madison. Preliminary results from a set of experiments currently in progress indicate that the olfactory system of salmon is very acute, and that they can discriminate between stream odors.

A set of field experiments must also be undertaken to furnish final proof of the hypothesis herein contained. Of a number of possible methods of solution, one promises to be of some practical value. The hypothesis could be tested by exposing salmon to a constant, artificial odor through the fingerling stage and then determining if the fish conditioned in a hatchery could be decoyed to a neighboring stream upon return from the sea. Should this be the case, it would aid in salvaging the declining salmon runs where new dams may obstruct passage to their parent streams.

Before field experiments can be realized, however, it is necessary to learn a great deal more about the olfactory responses in fish, and about the other systems which may be involved in migratory behavior.

Other field experiments are being planned which will be aimed toward measuring the preciseness and determining the means by which salmon choose one tributary over another.

SUMMARY

1. Various theories have been advanced to explain the mechanism by which migrating salmon return to their parent stream. One of these postulates the presence of some characteristic odor of the stream which guides the returning migrants. This theory presents two distinct problems:
   (1) Do streams have characteristic odors to which fish can react? If so, is the odor organic or inorganic in nature, or a combination of both?
   (2) Can salmon detect and discriminate between such odors, if they do exist?
2. In an attempt to answer the first question, a conditioned response training program was started with the bluntnose minnow. The fishes were able to discriminate successfully between chemical differences of two Wisconsin creeks after two months' training.
3. Extinction tests indicated that these minnows would respond to the stream odors after a "forgetting period," which was longer in fishes trained when young than in those trained in senility.
4. Heat cautery of the olfactory epithelium produced fish which were no longer able to respond to the training odors; proving that olfaction was the sole means of discrimination in these tests.
5. Chemical analysis of the stream waters indicated a total absence of CO_2; proving that this compound was not that which was detected.
6. Fractionation of the stream waters proved that the fish did not react to the inorganic ash, or to the distillate or residue of water fractionated at 100°C. They reacted to the distillate but not the residue, of water fractionated by vacuum distillation at 25°C.; a strong indication that the odorous stimulant is a volatile, aromatic substance.
7. Preliminary tests with salmon proved that they can detect the stream odors, and that they were able to discriminate between them.
8. It is postulated that the nature of the guiding odor must be such that it have meaning only for those salmon conditioned to it during their fresh-water sojourn. Any substance which was merely a general attractant could not guide salmon to their "home" tributary.

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STRUCTURAL HETEROZYGOSITY IN A VERY RARE SPECIES OF GRASSHOPPER[1]

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INTRODUCTION

Species of animals which are excessively rare or localized are interesting since they may provide critical evidence in connection with a number of problems in fundamental evolutionary theory. Such species are, however, of several different categories. On the one hand we have those which are restricted to some very small territory (*e.g.* an oceanic island) surrounded by a region which the species could not conceivably inhabit. The smallness of the distribution area in this case is not due to any inability of the species to adapt itself. On the other hand there are certain species which inhabit relatively minute areas of the earth's surface, to which they are apparently confined by their inability to adapt themselves to the ecological conditions of the surrounding terrain. Species of this type may be confined to a single such area or to several small areas between which the species does not occur.

Most very rare species show little obvious variation, or at any rate their populations are not visibly polymorphic, and it has been generally assumed, on theoretical grounds, that where the breeding population is of the order of only a few hundred individuals or less there will be a strong tendency towards genetic homozygosity. The present note deals with a case in which an extremely rare species shows a very high degree of structural heterozygosity in its chromosomes; we do not believe that any comparable case has been previously recorded.

Among the North American grasshoppers there are a number of species which have been collected at a single locality, or very few localities, and which are represented in collections by single individuals, or by very small series. Certain of these (such as *Spharagemon superbum* known only from two individuals collected at Katherine, Willacy Co., Texas) are forms which occur not far from the Mexican border, and may have an extensive distribution south of the border, where little collecting has been done. *Shotwellia isleta* is only known from two individuals, one from near Albuquerque, N. M., the other from Gomez Palacio, Durango, Mexico, approximately 700 miles to the south. It is at least possible that this species may be locally common in certain areas of Mexico that have not been visited by collectors.

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Such considerations do not apply in the case of species which occur in thoroughly collected areas of the United States, remote from the Mexican border. It is fairly certain that *Anconia caeruleipennis,* known only from the vicinity of Walker Lake, Nevada, is a species of extremely restricted distribution, and is probably represented by very few individuals at the present time. The Decticine katydid, *Zacycloptera atripennis,* also known only from Walker Lake, is in the same category. These species are almost certainly "relics" which had a wider distribution in Pluvial times, when the Great Basin area contained extensive lakes, represented at the present time only by a few widely scattered bodies of water, of which Walker Lake is one of the largest.

The present note deals with another very rare species of grasshopper, *Pedioscirtetes nevadensis* (subfamily Truxalinae). The species was described by Thomas (1873) from material collected during the 1871 Geographical Survey West of the 100th Meridian. The exact locality where the material was obtained is not known, but may have been in southeastern Nevada, although the species has never been subsequently recorded from that state. Bruner (1890) described *"Pedioscertetes" pulchella,* a synonym of *P. nevadensis,* from specimens collected on lava flows at Birch Creek, Idaho, apparently feeding on *Grayia polygaloides* (Chenopodiaceae). Ball *et al.* have recorded the species from near Springerville and near Flagstaff, Arizona. These are the only published records of *P. nevadensis,* but in addition Rehn and Hebard collected it on the Paunsaugunt Plateau, west of Bryce Canyon, Utah. None of these orthopterists obtained the species in large numbers and at each locality where it has been found the population is apparently very small.

The colony of *P. nevadensis* on which the present work was based was discovered by the senior author in a *Pinus ponderosa* forest on volcanic soil, approximately 8 miles northeast of Flagstaff. This may be the same locality at which the late E. D. Ball collected the species. Adults and nymphs were present July 24-25, 1949, but only adults were collected August 15, 1950. The population was restricted to a small clearing, most of the insects being found on the Boraginaceous plant *Lappula coronata* Greene, which seemed to constitute the main diet. Ball *et al.* (1942) have stated that *P. nevadensis* is closely associated with the composite *Actinia richardsoni,* but this plant was not present at the locality where our collection was made.

The known distribution of *P. nevadensis* thus embraces a large area of northern Arizona, Utah, Idaho and (possibly) southeastern Nevada, but within this area the species has been taken at only a very few widely scattered localities, in spite of much intensive collecting by experienced entomologists such as Rehn, Hebard, Tinkham, Ball and others. Several orthopterists have looked for the species in the Flagstaff area without success. We are therefore justified in regarding *P. nevadensis* as a relic species which survives precariously in a few suitable localities. The colony collected by the senior author probably does not consist of more than a few hundred individuals in any one year (twenty-five were taken in 1949, twelve in 1950, care being taken not to exterminate the colony). Under such circumstances a significant degree of inbreeding probably occurs, particularly since the species is somewhat sluggish in its movements. It was hence a complete surprise to find that *P. nevadensis* (or at any rate, this particular colony of it) exhibits a high degree of structural heterozygosity in certain of its chromosomes.

P. nevadensis is a rather isolated species taxonomically, the only other member of the genus, *P. maculipennis*, being not very closely related. *P. maculipennis* occurs on low, eroded hillsides in west Texas, southern New Mexico, southeastern Arizona and semi-desert areas of northeastern Mexico. Although a local species, the individual colonies usually show a high population-density, the species being frequently abundant in suitable environments.

The genus *Pedioscirtetes* is probably fairly closely related to *Acrolophitus*, and it is possible that a closer phyletic relationship exists between *Acrolophitus hirtipes* and *P. nevadensis* than between the latter and *P. maculipennis*.

The individuals in the Flagstaff population exhibit a certain amount of phenotypic polymorphism, but it is not known whether this has any genetic basis. The distinctness of the oblique cream-colored bands on the outer face of the hind femora is variable, and the dorsum of the pronotum, which is normally green, is tinged with purplish brown in some females. Bruner's types of *P. pulchella* had the tegmina mottled while in all our specimens they are uniformly green, but a similar variation occurs in *Acrolophitus hirtipes*, in which individuals from different localities may have the tegmina mottled or immaculate.

CYTOLOGICAL OBSERVATIONS

The cytology of *P. nevadensis* can best be understood after comparison with that of *P. maculipennis*. The latter species possesses a chromosome set which is of a type very characteristic of the sub-family Truxalinae, consisting of twenty-three acrocentric chromosomes in the male, that is, an X and eleven pairs of autosomes. The three smallest pairs of autosomes are considerably shorter than any of the others; since they are approximately the same size these three pairs cannot be distinguished from one another (fig. 1). The "short arms" of several of the autosomal chromosomes are quite conspicuous in various stages of mitosis and meiosis. Apart from the X, which is negatively heteropycnotic in the early spermatogonial divisions and again at the first meiotic metaphase, the chromosomes of *P. maculipennis* do not seem to contain extensive heterochromatic regions. Observations on resting stage nuclei and prophase stages of meiosis show that there is little heterochromatin in the autosomes. No structural heterozygosity was seen in nine individuals from Bottomless Lakes State Park, Chaves Co., New Mexico, the size of the three pairs of small chromosomes being apparently constant.

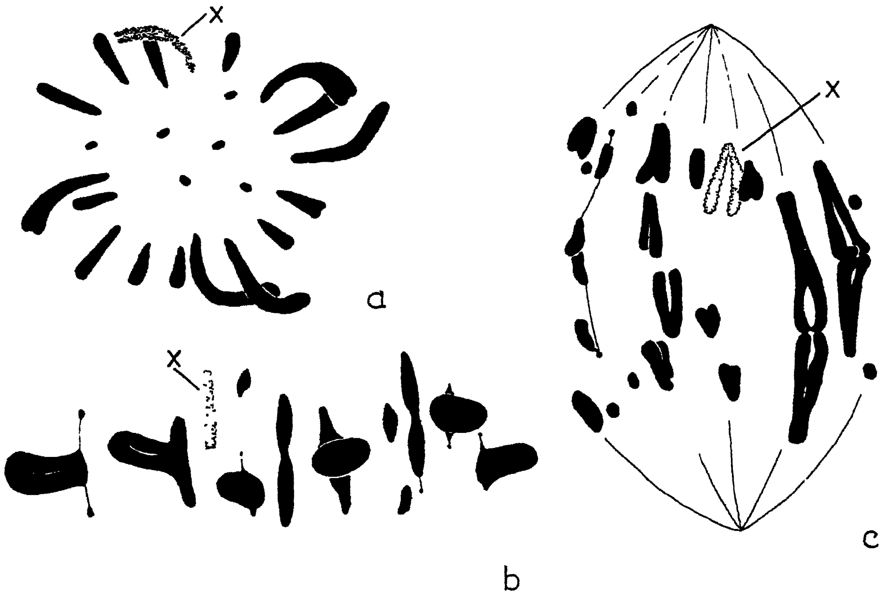

FIGURE 1. *Pedioscirtetes maculipennis: a,* spermatogonial metaphase; *b,* first metaphase; *c,* first anaphase.

P. nevadensis likewise has twenty-three chromosomes in the male, but the appearance of the three pairs of small chromosomes is quite different and varies from one individual to another. Each of these chromosomes appears to have acquired additional heterochromatin which is not present in *maculipennis.* The six small autosomes of *nevadensis* when seen in mitotic metaphase (fig. 2) are mostly V's or J's, considerably larger than the corresponding elements of *maculipennis,* but a variable number of them are small dots. At least two of these three autosomal elements may be represented either by a V or a dot. Thus the majority of individuals studied

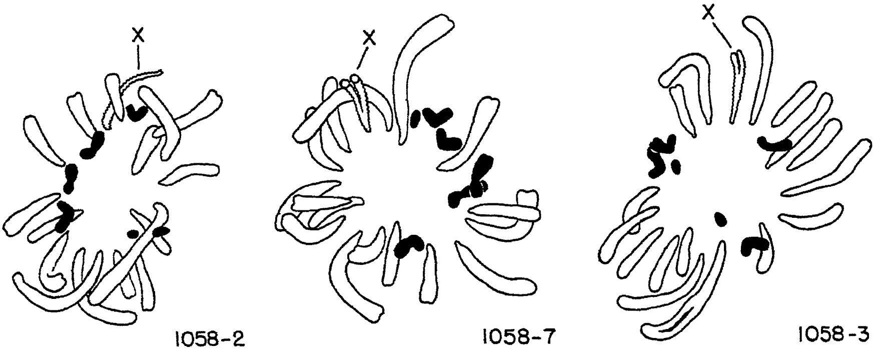

FIGURE 2. *Pedioscirtetes nevadensis:* spermatogonial metaphases of three different individuals. The six small autosomes are shown in black, the other autosomes in outline only.

had one or two of the three small pairs composed of elements of different sizes. Apparently, in such cases the smaller chromosome lacks a heterochromatic segment which is present in the larger one. Of nine individuals studied, six (nos. 1058-1, 1058-4, 1058-5, 1058-6, 1058-7 and 1058-9) had one of the three small pairs unequal (SL), the two other pairs being homozygous for the larger type of element (LL). We designate such individuals by the formula LL-LL-SL; it is not certain whether the SL bivalent is the same one in all six individuals. One grasshopper (1058-2) was a double heterozygote, *i.e.*, it had two unequal bivalents (formula: LL-SL-SL) and

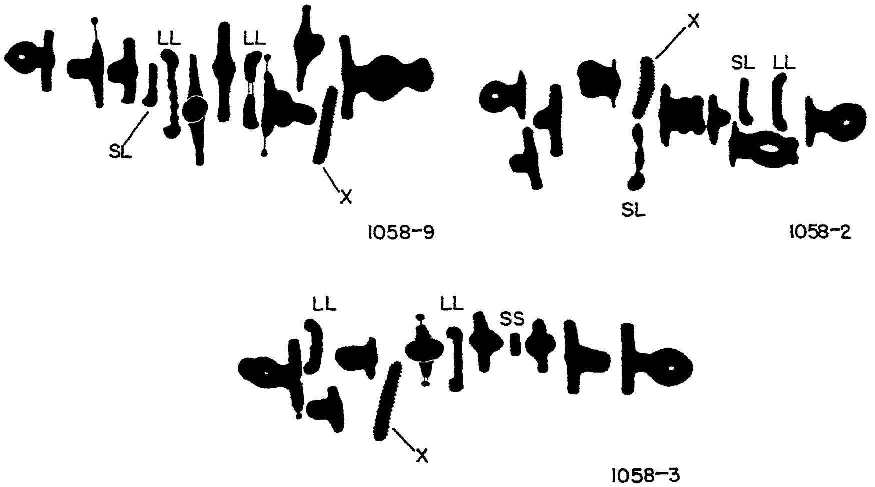

FIGURE 3. *Pedioscirtetes nevadensis:* first metaphases of three different individuals.

two (1058-3 and 1058-8) were structural homozygotes of the formula LL-LL-SS. The appearance of spermatogonial and meiotic divisions in these various types of individuals is shown in figs. 2–4. The "S" chromosomes are approximately one-third the size of the "L" chromosomes and seem to be effectively acrocentric. Thus the heterozygous bivalents are characteristically L-shaped and can be recognized before anaphase-separation has started.

Apparently the three small bivalents never form more than a single chiasma, regardless of whether they are structurally homozygous or heterozygous. Segregation of the S and L chromatids in unequal bivalents always takes place at the first meiotic division. This indicates that the cytological inequality is either situated proximal to the region in which the chiasma is formed or in the other arm of the chromosome.

That the small metacentric chromosomes of *P. nevadensis* do contain fairly extensive heterochromatic segments is clear from a study of resting primordial spermatogonia and early meiotic prophases, in which these heterochromatic segments can be clearly distinguished at certain stages. We believe that the "S" chromosomes lack certain heterochromatic seg-

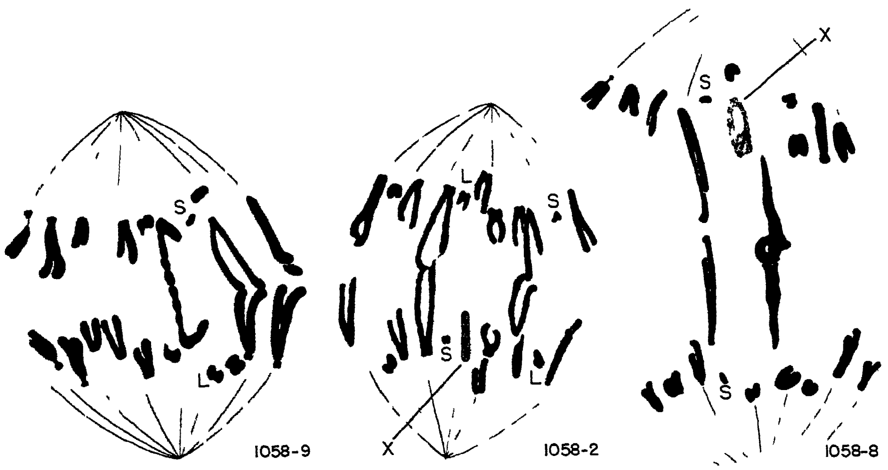

FIGURE 4. *Pedioscirtetes nevadensis:* first anaphases of three different individuals. In no. 1058-9 not all the chromosomes are drawn. In the case of the three small pairs of chromosomes the longer type is labelled L, the shorter type S.

ments present in the "L" chromosomes, but a detailed analysis is not possible with the available material.

We have made an attempt to compare the chiasma-frequencies of the two species of *Pedioscirtetes.* The results of this comparison are given in table 1. The difference in chiasma frequency between the two species is entirely due to the eight large bivalents, since the three small ones in both species invariably form a single chiasma.

DISCUSSION

Bivalents composed of two chromosomes of different sizes have been reported in a number of species of grasshoppers by Carothers (1913, 1931), Robertson (1915), Wenrich (1916), McClung (1928), Belar (1929), Hearne and Huskins (1935), Darlington (1936) and White (1951). Most of the species in which they have been recorded are widespread forms with large populations in which "drift" would not be an effective force. Moreover, in most of the cases previously studied, only a relatively small proportion of the population is heterozygous (*e.g.*, six out of a sample of fifty-six individuals of *Trimerotropis bilobata).* The present case is remarkable because of the very high

TABLE 1

CHIASMA FREQUENCIES OF THE TWO SPECIES

Number of chiasmata per nucleus	17	18	19	20	21	22	23	24	Mean
	Number of cells found								
P nevadensis	...	...	...	5	14	15	14	2	21.88 ±0.15
P. maculipennis	13	18	13	5	1	...	...	...	18.26 ±0.15

degree of heterozygosity present in a very small population. Although we did not attempt to determine the actual number of individuals present in the colony, we feel sure that it is so small that "drift" would inevitably have led to homozygosity unless rather strong selective forces were operating in favor of the heterozygotes. This implies, of course, that the various classes of homozygotes (or some of them) must be at a selective disadvantage. We did not find any LL-LL-LL individuals in the present sample. If the six single heterozygotes all belong to the same class (*i.e.*, if the unequal bivalent is the same one in all of them), then the absence of LL-LL-LL individuals from the sample is probably significant, and may indicate that they are of low viability. The extreme rarity of *P. nevadensis* is possibly due in part to the fact that it is a specialized feeder and in part to low fecundity (Ball *et al.* state that only six-eight eggs are laid in each "pod," compared with eleven-sixteen in *P. maculipennis*); but it may also result, to some extent, from a genetic mechanism involving lethality or near-lethality of certain categories of structural homozygotes.

Dobzhansky (1947, 1949, 1950—see also Dobzhansky, Burla and da Cunha, 1950) has put forward the view that adaptive polymorphism based on chromosomal rearrangements is especially characteristic of species having large effective breeding populations which are able to occupy a considerable variety of different ecological niches. Such a relationship may hold for the willistoni group of Drosophila and perhaps for some other groups of that genus. But in the present instance we have a species which is so rare that if it were a member of the genus Drosophila it would probably not have been discovered up to the present time exhibiting a high degree of chromosomal polymorphism. Of course, we have no proof that this polymorphism (which in any case is of quite a different type from the inversions of Drosophila species) is adaptive, but it seems almost inconceivable that it would have been preserved if it were not adaptive, in view of the population structure of the species. If we are able to collect *P. nevadensis* at other localities in the near future it will be most interesting to determine whether these other populations show the same type of structural heterozygosity as the one near Flagstaff.

SUMMARY

1. The very rare grasshopper *Pedioscirtetes nevadensis* has acquired certain heterochromatic segments in the three smallest pairs of autosomes which are not present in the corresponding autosomes of *Pedioscirtetes maculipennis.* These chromosomes, which are minute rods in the latter species, have been converted into much larger metacentric elements in the former species.

2. In *P. nevadensis* a large proportion of the individuals have one of these three pairs of chromosomes heterozygous for size, *i.e.*, one member of the pair carries a chromosome segment which is lacking in the other member. In some individuals a second pair of chromosomes is also heterozygous. We assume that this heterozygosity indicates the existence of a mechanism of heterosis of some kind.

3. The populations of *P. nevadensis* are of very small size, the species being one of the rarest North American grasshoppers. It is believed that this is the first account of cytological polymorphism in a species with such small populations.

4. The chiasma frequency of *P. nevadensis* is significantly higher than that of *P. maculipennis.*

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DEVELOPMENT OF HETEROSIS THROUGH NATURAL SELECTION IN EXPERIMENTAL POPULATIONS OF DROSOPHILA PSEUDOOBSCURA

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Most natural populations of *Drosophila pseudoobscura* are variable with respect to the gene arrangement in their third chromosomes. Several gene arrangements, differing in inversion of blocks of genes, coexist in many populations. Since the carriers of the different chromosomes interbreed freely, inversion heterozygotes and homozygotes are formed. The heterozygotes, provided that the two chromosomes with different gene arrangements are derived from the population of the same locality, are, as a rule, superior in adaptive value to the homozygotes (Dobzhansky, 1947a and b). This is true both in experimental and in natural populations (Wright and Dobzhansky, 1946; Dobzhansky and Levene, 1948). The adaptive superiority, heterosis, is however absent in some experimentally produced inversion heterozygotes which carry chromosomes derived from populations of geographically remote localities (Dobzhansky, 1949, 1950). The heterosis is, thus, not an intrinsic property of a gene arrangement. It is a result of interaction of complexes of polygenes carried in chromosomes with different gene arrangements. Within the population of each geographic region, the polygene complexes in the chromosomes have become mutually adjusted, or "coadapted," by a process of natural selection. The coadaptation results in a high fitness of the Mendelian population in which the chromosomes with the coadapted gene complexes occur.

The present article reports the results of the experiments which show that heterosis may indeed arise owing to the action of natural selection.

MATERIAL AND TECHNIQUE

The material used in this experiment consisted of twelve strains homozygous for the Chiricahua (CH), fifteen strains homozygous for Standard (ST), and twelve strains homozygous for the Arrowhead (AR) gene arrangements in the third chromosome. The CH strains were derived from wild ancestors collected in Chihuahua, Mexico, by Professor H. T. Spieth. The ST and AR strains came from the population of Piñon Flats, Mount San

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[1] Experimental data by Th. Dobzhansky, mathematical analysis by H. Levene.

[2] Research of H. Levene under contract with Office of Naval Research.

Jacinto, California. These are the same strains which were used in the experiments of Dobzhansky (1950), and were carried as laboratory stocks during the interim. The construction of the population cages, and the techniques of sampling the experimental populations have been described by Wright and Dobzhansky (1946) and Dobzhansky (1947b, 1950).

VIABILITY AND ADAPTIVE VALUE

We define heterosis as adaptive superiority of heterozygotes over homozygotes. In our experiments, the adaptive superiority of inversion heterozygotes over homozygotes is detected and measured by observing the rates of change in the frequencies of chromosomes with different gene arrangements in experimental populations. A population with known proportions of two gene arrangements is allowed to breed in a population cage; samples of the eggs deposited by the flies in the cage are taken at desired intervals, and the chromosomes are examined in the larvae which develop from these eggs. The relative frequencies of the gene arrangements undergo, in many experiments, rapid changes from generation to generation. The occurrence of such changes proves that the different chromosomal types are not equal in adaptive value. Now, if the carriers of one gene arrangement were adaptively superior to those with another gene arrangement, one would expect that the less well adapted arrangement should eventually be lost, and the population should become uniform for the better adapted chromosomal type. This, however, is not what is actually observed in most experiments. Instead of one gene arrangement crowding out the other, an equilibrium is eventually reached in the experimental populations, at which both gene arrangements continue to occur with certain frequencies. The attainment of equilibria is expected if the highest adaptive values occur in the inversion heterozygotes, while the homozygotes are inferior in adaptive value (Wright and Dobzhansky, 1946).

The relative viabilities of the chromosomal types are not necessarily proportional to their adaptive values, although the viability is evidently one of the important variables which determine the adaptive value of a genotype. In non-lethal genotypes, a deficient viability may be compensated for by other qualities, such as a greater fecundity or a greater sexual activity (Dobzhansky, 1950; Wallace, 1948; da Cunha, 1949). The viability of a chromosomal type between the egg and the adult stage is measured, in our experiments, by observing the differential survival (or differential mortality) of known chromosomal types under competition (Dobzhansky, 1947, 1950). Between two and three thousand flies heterozygous for two gene arrangements, ST and CH, are introduced in a population cage. According to the first law of Mendel, 50 per cent of the eggs deposited in such a population must be inversion heterozygotes, ST/CH, 25 per cent homozygotes ST/ST, and 25 per cent homozygotes CH/CH. Because of the very large number of eggs deposited in the population cages, the competition for food is very stringent among larvae in the population cages; less than 10 per cent, and perhaps less than 1 per cent, of the eggs deposited in the population cages produce adult flies. Now, if the mortality of the different genotypes is selective, the heterozygotes and the homozygotes may be more or less common among the adult flies which hatch in the population cages than they were among the eggs deposited by the parents. The relative viabilities of the genotypes are arrived at by comparison of the frequencies of the chromosomal types among the eggs deposited and among the adult flies which hatch in the population cages.

VIABILITY OF THE HYBRIDS BETWEEN STRAINS FROM CALIFORNIA AND FROM MEXICO

The Mexican Chiricahua strains ($\mathrm{CH}^{M}/\mathrm{CH}^{M}$) were outcrossed, in ordinary culture bottles, to Standard strains from California ($\mathrm{ST}^{C}/\mathrm{ST}^{C}$), or to California Arrowhead strains ($\mathrm{AR}^{C}/\mathrm{AR}^{C}$). On January 24, 1948, approximately 2300 $\mathrm{ST}^{C}/\mathrm{CH}^{M}$ hybrid flies were placed in population cage 55, and a like number of $\mathrm{AR}^{C}/\mathrm{CH}^{M}$ hybrids in cage 56. Both cages were kept at 25°C. The adult flies hatching in the cages in the next generation were isolated, and outcrossed in individual cultures to known CH homozygotes. The gene arrangement in the third chromosome was determined in six larvae in the progeny of each culture. This permits us to infer the chromosomal constitution of the parents which hatched in the cages. The results are summarized in table 1 (see also Dobzhansky, 1950).

Far from exhibiting a superior viability, the $\mathrm{AR}^{C}/\mathrm{CH}^{M}$ heterozygotes are quite significantly inferior to the $\mathrm{AR}^{C}/\mathrm{AR}^{C}$ homozygotes, and equal to, or even inferior, to the poorly viable $\mathrm{CH}^{M}/\mathrm{CH}^{M}$ homozygotes. The performance of the $\mathrm{ST}^{C}/\mathrm{CH}^{M}$ heterozygotes is different in the two sexes. Among the males, the homozygotes and heterozygotes do not differ significantly in viability. Among the females, the heterozygotes are decidedly inferior to the $\mathrm{ST}^{C}/\mathrm{ST}^{C}$ homozygotes, and about equal to the $\mathrm{CH}^{M}/\mathrm{CH}^{M}$ ones.

THE NATURAL SELECTION EXPERIMENT

If the adaptive values of the chromosomal types were proportional to the relative viabilities of their carriers, one could predict that the Mexican CH^{M} chromosomes would, given enough time, be eliminated by natural

TABLE 1

THE RELATIVE VIABILITY OF HOMOZYGOTES AND HETEROZYGOTES FOR CALIFORNIA AND MEXICAN CHROMOSOMES IN THE F_2 GENERATION OF THE INTERRACIAL CROSS

	Cage 55			Cage 56		
	$\mathrm{CH}^{M}/\mathrm{CH}^{M}$	$\mathrm{ST}^{C}/\mathrm{CH}^{M}$	$\mathrm{ST}^{C}/\mathrm{ST}^{C}$	$\mathrm{CH}^{M}/\mathrm{CH}^{M}$	$\mathrm{AR}^{C}/\mathrm{CH}^{M}$	$\mathrm{AR}^{C}/\mathrm{AR}^{C}$
Observed ♀♀	25	48	47	17	29	29
Viability	0.98 ± .25	1	1.87 ± .39	1.10 ± .35	1	1.91 ± .51
χ^2	0.01		9.10	0.10		5.96
Observed ♂♂	32	74	34	47	75	55
Viability	0.81 ± .18	1	0.86 ± .18	1.18 ± .23	1	1.39 ± .25
χ^2	0.97		0.50	0.77		3.29

selection in competition with the California ST^C and AR^C chromosomes. The experiment designed to test this prediction was started in the autumn of 1949. As before, the CH^M/CH^M strains were outcrossed in ordinary culture bottles to ST^C/ST^C or to AR^C/AR^C strains. On November 15th, some 2000 ST^C/CH^M hybrids were placed in cage 66 and AR^C/CH^M hybrids in cage 67. The cages were kept in an incubator at 25°C. From time to time samples of 300 chromosomes (150 larvae) were taken in each cage, as described before (Wright and Dobzhansky, 1946; Dobzhansky, 1950). The results are summarized in table 2 (see also figures 1 and 2 below).

TABLE 2

PERCENTAGE FREQUENCIES OF STANDARD (ST) AND ARROWHEAD (AR) CHROMOSOMES OF CALIFORNIA ORIGIN, AND OF CHIRICAHUA (CH) CHROMOSOMES OF MEXICAN ORIGIN IN THE POPULATION CAGES 66 AND 67.

	Cage 66		Cage 67	
	ST^C	CH^M	AR^C	CH^M
November 15, '49	50.0	50.0	50.0	50.0
M December '49	57.0	43.0	58.3	41.7
M January '50	63.7	36.3	61.7	38.3
L February '50	72.7	27.3	68.0	32.0
E April '50	76.7	23.3	66.3	33.7
M May '50	71.0	29.0	73.0	27.0
L June '50	72.0	28.0	76.6	23.3
L September '50	69.3	30.7	75.3	24.7
M November '50	64.3	35.7	76.0	24.0
M January '51	69.0	31.0	...	...

*E = early; M = middle; L = late.

In both experiments, the frequencies of CH^M chromosomes rapidly declined at first, while the frequencies of ST^C and AR^C increased. However, instead of elimination of the CH^M chromosomes, equilibria have become established at which the frequencies of the competing chromosomes have become stabilized. This is, of course, the outcome which would be expected if the ST^C/CH^M and AR^C/CH^M heterozygotes were heterotic. How are we to reconcile this result with the data (table 1) which suggest that the heterozygotes are less viable than the homozygotes?

The Working Hypotheses

Two working hypotheses may be suggested at this point. *The First Hypothesis.* The heterozygotes which carry the California and the Mexican chromosomes are always heterotic, that is, superior in adaptive value to the homozygotes. This would explain the establishment of the equilibria observed in cages 66 and 67. The inferior viability of the heterozygotes, observed in populations 55 and 56 (table 1), is compatible with this hypothesis. Indeed, the deficient viability may be overbalanced by other adaptively valuable qualities, which result in a net adaptive superiority of the heterozygotes.

The Second Hypothesis. The original ST^c/CH^M and AR^c/CH^M heterozygotes, obtained in the F_1 generation of the interracial cross, were not heterotic. Hence, a low viability of the heterozygotes was observed in populations 55 and 56. However, heterosis has developed in populations 66 and 67. The emergence of heterosis in these populations may have taken place if some combinations of California and Mexican chromosomes gave rise to heterotic genotypes, while others did not. In the population cages the genotypes that confer high adaptive values on their possessors are selected, and crowd out less well adapted genotypes. Two variants of the second hypothesis are possible; they will be presented in the Discussion.

Testing the Hypotheses

The two working hypotheses outlined above can be tested experimentally. If the first hypothesis is correct, the viability of the heterozygotes ST^c/CH^M and AR^c/CH^M formed in population cages 66 and 67 after the equilibria have become established must still be lower than the viability of the ST^c/ST^c and AR^c/AR^c homozygotes. If the second hypothesis is correct, the heterozygotes may have undergone improvement in cages 66 and 67, and their viabilities may now be higher than those of the homozygotes.

In October, 1950, about 40 pair matings were made from flies hatched in population cage 66. The gene arrangements were determined in 8 larvae from the offspring of each pair. Where necessary, other pair matings were made in the following generations. As a result, 10 strains homozygous for ST^c, and 10 homozygous for CH^M, were established. These new ST^c and CH^M strains were then intercrossed in ordinary culture bottles. On December 27, 1950, approximately 2500 ST^c/CH^M heterozygotes were placed in population cage 75, at 25°C. Among the adult flies hatching in this cage in the next generation, 200 females and 200 males were isolated and outcrossed in individual cultures to known ST/ST homozygotes. Six larvae in the progeny of each culture were examined for gene arrangement. The chromosomal constitution of the 400 parents is shown in table 3.

TABLE 3

THE RELATIVE VIABILITY OF HOMOZYGOTES AND HETEROZYGOTES FOR CALIFORNIA AND MEXICAN CHROMOSOMES AFTER A PROCESS OF NATURAL SELECTION

	Cage 75		
	CH^M/CH^M	ST^c/CH^M	ST^c/ST^c
Observed ♀♀	34	117	49
Viability	0.53 ± .11	1	0.78 ± .14
χ^2	9.95		1.99
Observed ♂♂	35	117	48
Viability	0.55 ± .11	1	0.76 ± .14
χ^2	9.20		2.32

The ST^c/CH^M heterozygotes now have a viability significantly superior to the homozygous ST^c/ST^c and apparently superior to CH^M/CH^M in both sexes. This was not the situation observed in 1948 (table 1). Heterosis has, indeed, developed in population cage 66. The second hypothesis is correct.

MATHEMATICAL ANALYSIS OF SELECTION EXPERIMENTS

If more than the roughest qualitative idea of what is occurring is desired, a mathematical model must be set up, and some attempt made to estimate the relevant parameters. The pertinent mathematical model, and a method of estimating the adaptive values by the method of least squares, have been furnished by Sewall Wright (Wright and Dobzhansky, 1946). The method of Wright, like any other statistical method, has certain limitations. Among these, in the present case, are the lack of an error estimate, the occasional obtaining of biologically impossible negative adaptive values, and the requirement that the adaptive values remain constant during the period under consideration.

In cage 66 the adaptive values have almost certainly changed during the course of the experiment. To meet this need a simple method has been devised which may be used for analyzing such data and which also gives some estimate of the possible range of the true adaptive values. In a later paper, it is hoped to give a more precise investigation of methods for finding adaptive values.

Samples were taken from cages 66 and 67 at varying intervals, each of which was greater than a generation. (The length of a generation under those conditions is 25 days.) These intervals will hence be called periods rather than generations. Table 4 gives the results.

Wright (Wright and Dobzhansky, 1946) has given formulas for the change in $\tilde{q}$ from one generation to the next, where $\tilde{q}$ is the fraction of CH chromosomes in the population. Haldane (1932) has pointed out that formulas for selection take a simpler form in terms of $\tilde{x} = \tilde{q}/(1 - \tilde{q})$, the ratio of CH chromosomes to the other chromosomes in the population. (For some parts of the later analysis it is desirable to take the ratio of the less frequent to the more frequent chromosome, so that $\tilde{x}$ is less than one.) The sample estimate of $\tilde{x}$ is x, the ratio of the number of CH chromosomes to the number of the other chromosomes in a sample. The variance of x is approximately $x(1 + x)^2/n$, where n is the number of chromosomes sampled. In figures 1 and 2, x is plotted against time for cages 66 and 67. The plot is semilogarithmic because the graph should then be closer to a straight line for constant adaptive values. The middle solid line connects values of x, while the other two solid lines connect values of $x \pm 1.96\sigma_x$. The true values of $\tilde{x}$ in the cage might easily lie anywhere within the resulting band, or even somewhat outside of it at a few points. In the long run, 5 per cent of the plotted $x \pm 1.96\,\sigma_x$ will fail to include the true $\tilde{x}$.

We now come to the calculation of adaptive values. We will take the adaptive value of heterozygotes as 1, of CH homozygotes as W_1, and of the

TABLE 4

RESULTS OF SELECTION IN CAGE 66, STARTED WITH CH^M/ST^C HETEROZYGOTES, AND IN CAGE 67, WITH CH^M/AR^C HETEROZYGOTES. EACH SAMPLE CONTAINED 300 CHROMOSOMES. FURTHER EXPLANATION IN THE TEXT.

Period number	Elapsed time in days	Length of period in generations t	Number of CH	Percentage of CH	Ratio of CH to ST or AR x	Standard error σ_x	Adjusted change in x r	Coefficients for finding adaptive values	
								a	b
				Cage 66					
0	0		150	50.0	1.000	0			
1	30	1.20	129	43.0	.754	.063	.791	.265	1.265
2	60	1.20	109	36.3	.571	.050	.792	.508	.952
3	100	1.60	82	27.3	.376	.038	.771	.727	.741
4	140	1.60	70	23.3	.304	.033	.876	.765	.429
5	180	1.60	87	29.0	.408	.040	1.201	.528	.253
6	220	1.60	84	28.0	.389	.039	.970	.623	.421
7	310	3.60	92	30.7	.442	.042	1.036	.576	.375
8	360	2.00	107	35.7	.554	.049	1.120	.451	.395
9	420	2.40	95	31.7	.463	.043	.928	.523	.597
				Cage 67					
0	0		150	50.0	1.000	0			
1	30	1.20	125	41.7	.714	.060	.755	.324	1.324
2	60	1.20	115	38.3	.622	.054	.891	.408	.802
3	100	1.60	96	32.0	.471	.044	.840	.568	.740
4	140	1.60	101	33.7	.507	.046	1.048	.483	.449
5	180	1.60	81	27.0	.388	.039	.845	.676	.601
6	220	1.60	70	23.3	.304	.033	.860	.775	.451
7	310	3.60	74	24.7	.327	.035	1.021	.675	.298
8	360	2.00	72	24.0	.316	.034	.982	.691	.333

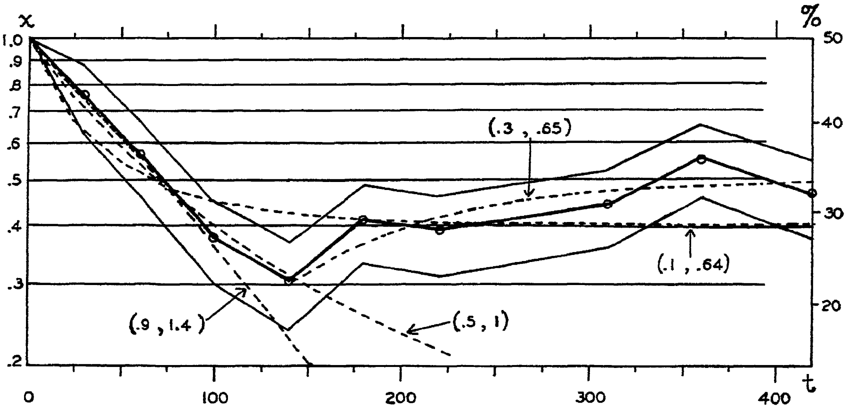

FIGURE 1. Results of cage 66. Vertical axis gives ratio of CH^M to ST^C (on left scale), percentage CH^M (on right scale). Horizontal axis time in days. Circles are sample values, upper and lower solid lines give 95 per cent confidence limits for true values. Dotted lines give theoretical changes if the adaptive values indicated were true.

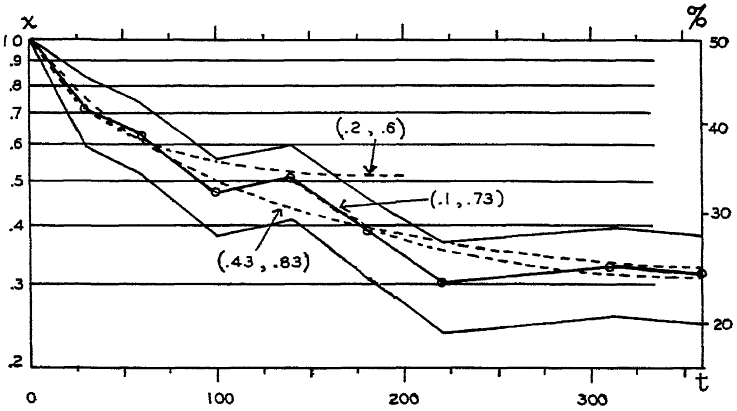

FIGURE 2. Results of cage 67. Vertical axis gives ratio of CH^M to AR^C. Remainder of explanation same as in Fig. 1.

other homozygotes as W_2. If the Hardy-Weinberg equilibrium holds at the egg stage, CH/CH, CH/ST, ST/ST occur in the proportions $\tilde{x}_0^2 : 2\tilde{x}_0 : 1$. After selection the proportions become $W_1\tilde{x}_0^2 : 2\tilde{x}_0 : W_2$, and in the next generation $\tilde{x}_1 = (W_1\tilde{x}_0^2 + \tilde{x}_0)/(\tilde{x}_0 + W_2)$, or

$$\tilde{x}_1/\tilde{x}_0 = (W_1\tilde{x}_0 + 1)/(\tilde{x}_0 + W_2). \tag{1}$$

The ratio $\tilde{x}_1/\tilde{x}_0$ changes more slowly from one generation to the next than does $\tilde{x}$ itself; it is for this reason that a semilog plotting is used in figures 1 and 2. The dotted lines in these figures are the result of applying (1) for certain W's discussed below. If the actual time interval from $\tilde{x}_0$ to $\tilde{x}_1$ is t generations instead of one, equation (1) will no longer serve. If the dotted lines in figures 1 and 2 were actually straight lines it could easily be shown that the correct formula would be

$$\left(\frac{\tilde{x}_1}{\tilde{x}_0}\right)^{1/t} = \frac{W_1\tilde{x}_0 + 1}{\tilde{x}_0 + W_2} \tag{2}$$

This formula is the best simple approximation to the true one and will be used as the basic formula. Its validity depends on the essential linearity of short portions of the dotted lines. The observed values of the left hand member of (2) are denoted by r in table 4.

Formula (2) can be solved for W_2, giving

$$\begin{gathered} W_2 = \tilde{a} + \tilde{b}\, W_1, \text{ where} \\ \tilde{a} = \left(\frac{\tilde{x}_0}{\tilde{x}_1}\right)^{1/t} - \tilde{x}_0, \text{ and} \\ \tilde{b} = \tilde{x}_0\left(\frac{\tilde{x}_0}{\tilde{x}_1}\right)^{1/t}. \end{gathered} \tag{3}$$

We thus see that knowledge of what occurs during a single period does not give us the separate values of W_1 and W_2, but only a linear relation between them. Given any two periods, not necessarily consecutive, we obtain two such equations, which can be solved to give W_1 and W_2. This can be done algebraically, or by plotting the two lines and finding the point of intersection. When this is done with actual sample data it often happens that the point of intersection involves a negative value of W_1, W_2 or both. This is biologically impossible, and means that no positive pair of W_1, W_2 could exactly reproduce the observed changes. This discrepancy could be due to either sampling error or an actual change in one or both W's. The lines from a number of periods can be plotted on the same graph, and one can then see by inspection which periods are consistent with each other and what values of W_1 and W_2 are likely.

Examination of figures 1 and 2 suggests that periods 1–4 of cage 66, 5–9 of cage 66, 1–4 of cage 67 and 5–8 of cage 67 are comparable. The corresponding lines from formula (3) are plotted in figures 3–6 and the values of a and b are given in table 4. Before further discussing figures

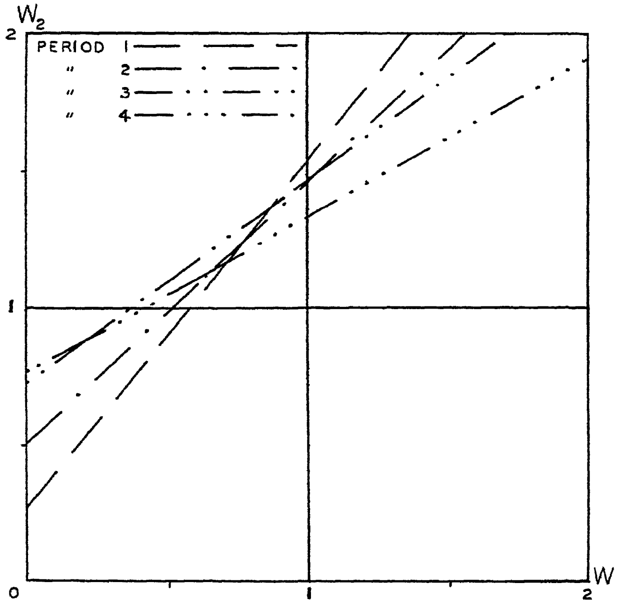

FIGURE 3. Adaptive values for early periods of cage 66. Any pair W_1, W_2 lying on a given line are compatible with the changes during the corresponding period.

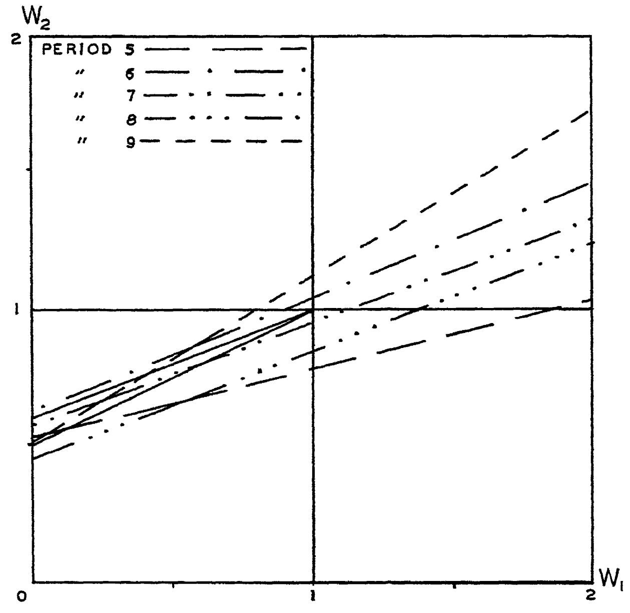

FIGURE 4. Adaptive values for later periods of cage 66. Any pair W_1, W_2 lying on the lower solid line are compatible with equilibrium at $\bar{x} = .5$, any pair on the upper solid line with $\bar{x} = .4$. Interpretation of broken lines same as in Fig. 3.

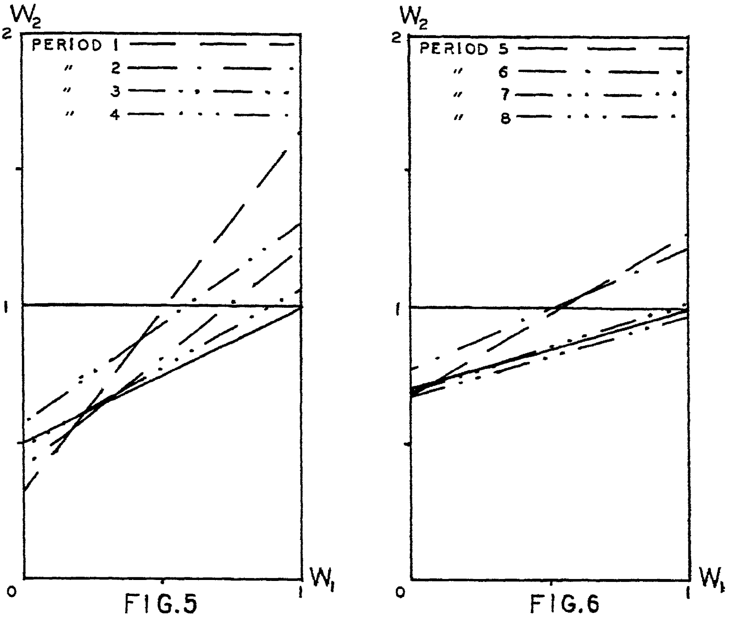

FIG.5 FIG.6

FIGURES 5 AND 6. Adaptive values for cage 67. Interpretation same as in Figs. 3 and 4. Solid line in Fig. 5 for $\bar{x} = .5$, solid line in Fig. 6 for $\bar{x} = .3$.

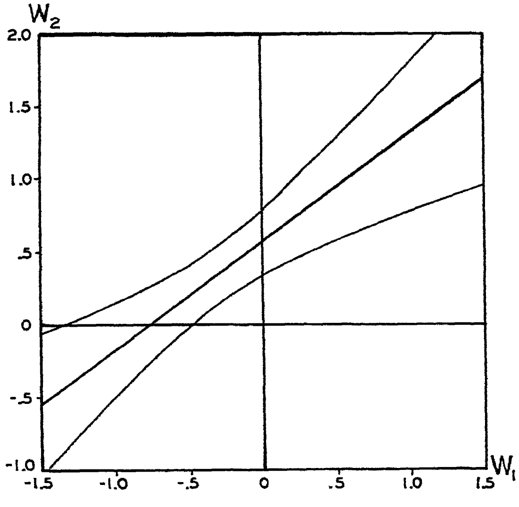

FIGURE 7. Limits of error for line of period 3, cage 67. For interpretation see text.

3–6, the sampling error of these lines will be discussed. Inspection indicates that the line for period 3 in cage 67 is incompatible with the other lines in figure 5 and accordingly this line is chosen for an illustrative example. In figure 7 the line $W_2 = a + bW_1$ corresponding to the observed x's is shown, together with a hyperbola that contains it. If a corresponding figure were drawn for a large number of different samples, the true $W_2 = \tilde{a} + \tilde{b}W_1$ corresponding to the true $\tilde{x}$'s would be contained entirely within the two branches of the hyperbola in 95 per cent of all cases. In our particular figure we can then say that any line drawn in such a way as not to cross either branch of the infinitely extended hyperbola could be the true line at the 95 per cent confidence level. The formula for the hyperbola is too complicated and of insufficient importance to give here, and the calculations are so lengthy that they were only done for this one case; however, all samples contained 300 chromosomes and had roughly the same error, so that the other lines calculated would have an error of the same order of magnitude as in figure 7.

With this indication of the uncertainty of the computed lines, we will proceed to study figures 3–6. Periods 1–3 of cage 66 (fig. 3) intersect in the neighborhood of $W_1 = .9$, $W_2 = 1.4$. (For brevity we will hereafter refer to this as the point (.9, 1.4,) etc. The theoretical series of values of $\tilde{x}$, starting with $\tilde{x} = 1$, resulting from this W_1 and W_2, can be calculated by formula (1), and are plotted as a dotted line in figure 1, taking the interval between generations as 25 days. This theoretical line is in good agreement with the observed for periods 1–3, but thereafter decreases rapidly, ultimately approaching $\tilde{x} = 0$. Period 4 intersects with 1 and 2 near (.5, 1), and the corresponding theoretical line is surprisingly enough in good agreement with the observed for all four periods, although thereafter it also tends toward $\tilde{x} = 0$.

Turning now to periods 5–9, figure 4 shows that many of the lines are nearly parallel and fail to cross, while the observed intersections are at widely separated places. Study of figure 1 shows that this is to be expected, since x fluctuates in an irregular manner. The main conclusion from figure 1 is that x has reached an equilibrium somewhere between .4 and .5 with superimposed chance fluctuations. Equilibrium can only occur with W_1 and W_2 both between 0 and 1; for equilibrium at $\bar{x}$, W_1 and W_2 must lie on the straight line between the points $(0, 1 - \bar{x})$ and (1, 1). The lines corresponding to $\bar{x} = .4$ and $\bar{x} = .5$ are shown as solid lines in figure 4. Considering only periods 5–9, there seems to be an increase from an original value of x = .3 to a final equilibrium at approximately .5. From figure 4, the most reasonable W values giving this $\bar{x}$ are (.3, .65). The corresponding theoretical line for $\tilde{x}$ is given in figure 1.

An attempt was also made to fit a single pair of W values to the entire experiment by a somewhat different method. For the entire experiment there seems to be an initial rapid drop followed by fluctuation about an equilibrium at about .4. The line for $\bar{x} = .4$ in figure 4 gives the possible pairs W_1, W_2 that result in this equilibrium. The smaller W_1 is chosen on this line, the more rapid is the early decline in $\tilde{x}$. Trial and error then gives $W_1 = .1$, $W_2 = .64$ as the pair giving the best over-all fit with the observed values, and the corresponding theoretical line is shown in figure 1. The fit is as good as can be expected, considering the fluctuations observed, but much of the theoretical line lies outside the confidence band. On the other hand the pairs (.5, 1.0) for periods 1–4 and (.3, .65) for periods 5–9, give the theoretical lines which lie wholly within the confidence bands, and it hence seems likely that a change in adaptive values occurred.

While the assumption of constant W's for the first four periods and a different set of constant W's for the remaining periods gives a good fit, this is not to be taken as proof of an abrupt change in adaptive values at the end of the fourth period. A gradual change is much more likely. Unfortunately we do not have enough information to see exactly when the change in W took place, or how long it took. The two sets of W's given above give merely the simplest interpretation of the data.

Turning now to cage 67, the first four periods in figure 2 seem to be leading to an equilibrium at $\bar{x} = .5$. In figure 5 the lines for periods 1, 2 and 4, and for $\bar{x} = .5$ intersect near (.2, .6) and the corresponding theoretical $\tilde{x}$'s are in good agreement with the first four periods. The line for period 3 in figure 5 is apparently incompatible with (.2, .6) but figure 7, already discussed, shows that it is quite possible for the true line for period 3 to pass through this point. During periods 5 to 8 a further drop occurs, to an apparent equilibrium near $\bar{x} = .3$. Figure 6 contains the lines for these periods and this $\bar{x}$. Periods 7, 8 and $\bar{x} = .3$ are compatible with any W_1 from 0 to 1 and any W_2 from .7 to 1. Period 5 suggests a low value of W_1 and hence the pair (.1, .73) was tried. The corresponding theoretical values of $\tilde{x}$, starting with $\tilde{x} = .5$ at the beginning of period 5, give an excellent fit. Again for this cage an attempt was made to find a single pair of W's that would serve for the whole experiment. Periods 1, 2, 7, 8, and $\bar{x} = .3$ all intersect near (.43, .83). The resulting theoretical $\tilde{x}$ line lies almost wholly within the confidence band, and hence, for this cage, the results can be accounted for by a constant pair of adaptive values, although naturally a better fit is obtained by using one pair for the early part and another pair for the later part of the experiment.

MATHEMATICAL DISCUSSION OF VIABILITY EXPERIMENTS

We turn now to the viability experiments. Suppose eggs are laid in a cage in the proportions 1 CH/CH : 2 CH/ST : 1 ST/ST. After selection the adults are classified as to genotype. There is a certain probability of misclassification; suppose the combination of selection and misclassification results in probabilities α, β, and γ that an adult be classified as CH/CH, CH/ST, and ST/ST and that the numbers actually recorded are a, b, and c. Suppose the viabilities of the three types be taken as $\tilde{V}_1$, 1 and $\tilde{V}_2$. (The letter V is used since the viability V is only one component of the adaptive value W discussed above.) Then the initial proportions 1:2:1 become $\tilde{V}_1 : 2 : \tilde{V}_2$ after selection. If six larvae are examined in classifying each adult, there is probability 1/64 that a given heterozygote will be classified CH/CH, and 1/64 that it will be classified ST/ST. Hence the probabilities of being listed in each of the three classes α, β and γ become $(32\ V_1 + 1)/d$, $62/d$, $(32\ V_2 + 1)/d$, where $d = 32(V_1 + V_2 + 2)$. In the special case of equal viabilities, $V_1 = V_2 = 1$, and $\alpha = \gamma = 33/128$, $\beta = 62/128$.

If sampling is continued until a fixed number b of heterozygotes have been obtained, a will have a Pascal distribution, with mean $b\alpha/\beta$ and variance $b\alpha(\alpha + \beta)/\beta^2$. [The Pascal distribution is discussed in section 11.3 of an excellent new book on probability and its applications to genetics, (Feller, 1950)]. To test the hypothesis that $\tilde{V}_1 = 1$, we then have for large samples

$$\chi^2_L = \frac{(a - b\alpha/\beta)^2}{\sigma^2_a} = \frac{(62a - 33b)^2}{3135b}, \tag{4}$$

with χ^2_L distributed as χ^2 with one degree of freedom. Dobzhansky (1950) used a formula for χ^2 which was supplied by his present coauthor, who assumed, without investigation of the matter, that a would have a Poisson distribution. That formula was erroneous, all computed χ^2's being too small by a factor of $62/95 = .6526$. Luckily this error does not affect the conclusions reached by Dobzhansky, all but one of his "significant" chi squares remaining significant.

If, on the other hand, sampling is continued until a fixed number of flies have been classified, for the study of $\tilde{V}_1$ we can proceed as if a comes from a binomial sample of a + b observations, with the probability of CH/CH being $(32\ \tilde{V}_1 + 1)/(32\ \tilde{V}_1 + 63)$. We then have the statistic

$$\chi^2_D = \frac{(a - Ea)^2}{Ea} + \frac{(b - Eb)^2}{Eb} = \frac{(62a - 33b)^2}{2046\ (a + b)} \tag{5}$$

This statistic was suggested by Dr. Everett R. Dempster, who pointed out that the formula in Dobzhansky (1950) was incorrect. Continuing our analysis for the binomial case, setting a equal to its expected value $(a + b)(32\ \tilde{V}_1 + 1)/(32\ \tilde{V}_1 + 63)$ and solving for $\tilde{V}_1$, we obtain the sample estimate

$$V_1 = \frac{62a}{32b} - \frac{1}{32}, \tag{6}$$

which is identical with the estimate in Dobzhansky (1950). Thus the two assumptions of constant b or constant a + b lead to the same estimate V_1. The two assumptions lead to quite different variances for a; however V_1 depends only on a/b, and this ratio has the same large sample variance in the two cases. The true variances depend on the unknown probabilities α, β and γ, but in practice they must be replaced by the sample values a, b and c, and this is valid in large samples. It can be shown that, under either mode of sampling, the sample estimate V_1 is approximately normally distributed with mean $\tilde{V}_1$ and variance

$$\sigma^2_{V_1} \cong \frac{(32V_1 + 1)\ (32V_1 + 63)}{(32)^2 b} \cong \left[\frac{62}{32}\right]^2 \left[\frac{a(a + b)}{b^3}\right] \tag{7}$$

We can now propose two additional chi square formulas, $\chi^2_V = (V_1 - 1)^2/\sigma^2_{V_1}$ and $\chi^2_a = [a - 33(a + b)/95]^2/[ab/(a + b)]$. All four of these formulas are valid in very large samples and would give equivalent results. However, in moderate sized samples they give different results. As an example, in cage 55 (table 1) for ST/ST females, where V is greater than one (V = 1.866), we find $\chi^2_V = 5.0$, $\chi^2_a = 8.2$, $\chi^2_D = 9.1$, $\chi^2_L = 11.8$. On the other hand in cage 75 (table 3) for CH/CH males, where V is less than one (V = .548), the relationships are reversed, and $\chi^2_V = 16.2$, $\chi^2_a = 11.7$, $\chi^2_D = 9.2$ and $\chi^2_L = 7.8$. The relative order of magnitude of the four chi squares should be the same as this in general, reversing as V is greater or less than one. All these chi squares are approximations valid in large samples; χ^2_V and χ^2_a should require larger samples to be satisfactory than do the other two, hence one of the other two should be used. It would probably be legitimate to use χ^2_D for $V < 1$ and χ^2_L for $V > 1$; however, this raises certain unsolved problems, and for the present χ^2_D is recommended, as a compromise for routine use. Values of χ^2_D for the present data are given in tables 1 and 3. The statistic χ^2_D for V_1 and the corresponding χ^2_D statistic for V_2 are logically independent, since the viability of one homozygote relative to the heterozygote need not affect the viability of the other heterozygote. On the other hand the two chi-squares are not independent statistically; *i.e.* the sum of the two may not be taken as a chi-square with two degrees of freedom. This comment applies to the other chi-squares discussed above.

There is another aspect to the use of χ^2_V. This amounts to using V with its approximate standard error from (7). This has the great advantage that we can test the hypothesis that $\tilde{V}$ has any given value. Furthermore an approximate confidence interval for $\tilde{V}$ can be given in the usual way as $V \pm C\sigma_V$, where C is 1.96, 2, 3, etc., as desired. These confidence intervals serve for most practical purposes. However, if greater accuracy is desired, particularly in small samples or when V is far from 1, we can consider a as a binomial variable from a sample of a + b observations. Then upper and lower confidence limits may be obtained for the expected value of a (see e.g. Fisher and Yates, 1943) and these two values of a substituted into (6) to obtain more accurate confidence limits for $\tilde{V}$. As a rule the length of the confidence intervals obtained in these two ways are about the same, but the limits by the more accurate method are both somewhat higher. For example, in the two cases used in the previous paragraph the standard error gives $(.33 < \tilde{V} < .77)$ and $(1.10 < \tilde{V} < 2.63)$ with 95 per cent confidence coefficient, while the more precise method gives $(.35 < \tilde{V} < .79)$ and $(1.23 < \tilde{V} < 2.82)$. Tables 1 and 3 give the values of V and their standard errors for the present data.

CONCLUSIONS FROM THE STATISTICAL ANALYSIS

We now turn to a consideration of what actually happened in cages 66 and 67. The strains used in cage 66 were tested for viability in cage 55 before cage 66 was started, and strains recovered from cage 66 during period 8 were tested for viability in cage 75. Initially, in cage 55, $V_1 = .98 \pm .25$ and $V = 1.87 \pm .39$ for females. while for males $V = .81 + .18$

and $V_2 = .86 \pm .18$. There was a significant difference between males and females. Assuming that adaptive value depends only on viability and depends equally on males and females, we would guess the adaptive values as $W_1 = .87$ and $W_2 = 1.26$. Periods 1–3 of cage 66 gave estimates $W_1 = .9$, $W_2 = 1.4$ which are in excellent agreement. During the later periods of cage 66 we estimated the adaptive values as $W_1 = .3$, $W_2 = .7$ with considerable margin for error. The flies derived from this cage gave viabilities of about $V_1 = .54$ and $V_2 = .77$, essentially the same for males and females. Again the agreement is fairly good, considering the variability of both the W's and V's. It seems almost certain that both the adaptive value and viability of ST homozygotes declined relative to the heterozygotes, and there is some indication that the same happened for CH homozygotes. The adaptive values and viabilities are in fairly good agreement.

The situation is somewhat different for cage 67. The course of events here could be explained either by adaptive values (.2, .6) for the early period and (.1, .73) for the later period, or by constant adaptive values (.43, .83). Viability was tested only before the selection experiment, so we cannot see whether the viability changed. In cage 56 the viability values were $V_1 = 1.1$, $V_2 = 1.9$ for females and $V_1 = 1.2$, $V_2 = 1.4$ for males, with an average value for both sexes of $V_1 = 1.2$, $V_2 = 1.5$. Study of figure 5 shows that these values, taken as adaptive values, would be compatible with periods 2 and 3, or even with periods 1, 2 and 3 remembering the variability shown in figure 7, in spite of the tremendous deviation from the best guess from figure 5. Hence in this cage also we might suppose adaptive values and viability are nearly equal, and have changed during the course of the experiment, but it seems more likely that adaptive values and viability are quite different; and the adaptive value may have remained unchanged, although it might equally well have changed a good deal.[3]

DISCUSSION

The development of heterosis may be visualized in two ways. First, it may be due to selection of coadapted third chromosomes. Secondly, it may involve selection of whole new genotypes. It should be kept in mind that the ancestral population of cage 66 included 15 different California and 12 different Mexican strains. Therefore, at least 15 different ST^C and 12 different CH^M chromosomes were involved, and probably considerably more since each "strain" may have carried up to four or more different chromosomes (two descended from the wild female progenitor and two or more from the male or males with which she mated before capture). It is possible, then, that some of the California chromosomes introduced into cage 66, or formed there by crossing over, gave heterosis with some of the CH chromosomes from Mexico. Such "heterotic" chromosomes would then be multiplied by selection, at the expense of the chromosomes which gave poorly viable heterozygotes. On the other hand, it is possible that heterosis arises through interaction between the genes located in the third chromosomes and genes in other chromosomes. In other words, the heterosis exhibited by the ST/AR, ST/CH, and AR/CH heterozygotes in the California populations may be the property of the genotype of the California race. Now, countless new genotypes were doubtless formed in the cages 66 and 67 by recombination of the California and the Mexican genotypes. Among these recombination products, some of the ST^c/CH^M heterozygotes which also carried certain constellations of the California and Mexican gene blocks, may have exhibited superior adaptive values. Such adaptive genotypes would be multiplied and established by natural selection.

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[3] No account has been taken in this analysis of the possibility of genetic drift. The size of the cage populations is much larger than the sample size, but there may still be at least some error due to drift. It is hoped to consider this aspect in a later paper.

The data at hand do not permit discrimination between these possible mechanisms of the development of heterosis. It may, nevertheless, be pointed out that the second mechanism would entail selection in our experiments of previously non-existent but adaptively coherent genotypes, which might differ both from the California and from the Mexican races in many genes. The greater the number of the gene differences involved, the less frequent will be the origin of the new genotype by recombination, and, consequently, the less easily repeatable will become the process. Evolutionary changes become less and less easily repeatable as they involve more and more gene substitutions in adaptively integrated genotypes.

SUMMARY

Hybrids of the California and the Mexican races of *Drosophila pseudoobscura* were kept in population cages for approximately 15 generations. A preliminary study of viability, and the early course of the population in the cages, showed no heterosis for the heterozygotes which carried a California and a Mexican third chromosome. Heterosis has, however, developed during the experiment, as indicated by the attainment of equilibrium and by a study of the viability of flies derived from the cage. Both tests gave statistically significant results. Methods were developed for estimating adaptive values and viability in such experiments, and for investigating the possible range of values of these constants consistent with the sampling error involved.

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PYLE-BLACK PLUMAGE IN THE FOWL

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Sexually dimorphic and dichromatic zoning of red-gold pigmentation is manifested in plumage of the Red Pyle Game. It occurs on a colorless (white) background due to inhibition of black by gene *I* (dominant white). The Red Junglefowl, Black-breasted Red Game, and Brown Leghorn illustrate pyle-zoning on a black background. Breeding experiments indicate that pyle-zoning is attributable to partial restriction of a specific black pigment by gene e^+, the wild type allele at the *E* (extended black) locus (*cf.* Smyth and Bohren, 1949). Expression of pyle-zoning is complete, fractionated, reduced, or absent (phenocryptic) dependent upon modifying polygenic complements.

Stippling is associated with an autosomal gene *(Sg)* which restricts black within feathers of juvenile plumage in both sexes; with the exception of certain feather groups in males of some Red Junglefowl biotypes, and of Light Brown Leghorns, expression of stippling is limited to female adult plumage. Genotype in wild type plumage is conceived to be $(e^+ e^+ Sg\ Sg)$. Segregants of Brown Leghorn ♂ $(e^+ e^+ Sg\ Sg)$ × White Minorca ♀ $(E\ E\ sg\ sg)$, and of Golden Campine ♂ $(e^+ e^+ sg\ sg\ Bg\ Bg)$ × Brown Leghorn ♀ $(e^+ e^+ Sg\ Sg\ bg\ bg)$, have been isolated and studied. Such fowls carry pyle-zoned black plumage in both sexes, incident to the genotype $(e^+ e^+ sg\ sg\ bg\ bg)$. Black in these birds is comparable to recessive black mammalian pelage, and has been reported previously by Goodale (1926), and by Serebrovsky (1926).

Pyle-black ♂ × Brown Leghorn ♀ produced F_1 progeny with males indistinguishable from the leghorn phenotype, and females characterized by incomplete, apico-marginal patterning of stippled feathers. Stippling *(Sg)* and autosomal barring *(Bg)* have been found epistatic to black pigment of pyle-black $(e^+ e^+)$ plumage; whereas, both secondary pattern genes are hypostatic to black pigment of fowls carrying extended black *(E E)* plumage, *e.g.*, Black Minorca and Black Langshan. A similar situation prevails for penciling *(Pg)*, *e.g.*, Partridge Rock and Dark Cornish, as suggested by studies now in progress.

Genetically and physiologically distinct forms of black pigment coincide with the genotypes $(e^+ e^+)$ and *(E E)*. Wild type and immediately derived plumage patterns (involving mutated secondary pattern factors) thus far tested are genetically $(e^+ e^+)$. Complete or modified pyle-zoning occurs in all junglefowls, associated with secondary patterns such as stippling, lacing, or barring. Examination of junglefowl and hybrid junglefowl skins in the American Museum of Natural History collection was made possible through the courtesy of Mr. Jean Delacour. Phenotypes observed were compatible with the view that all junglefowls are of the genotype (e^+ e^+). It appears that recognition of wild type (e^+) black is fundamental in significance for plumage genetics, classification of plumage patterns, and for identification of secondary pattern factor alleles as dominant or recessive. Wild type (e^+) black would seem to be the rational standard of reference, rather than extended (E) black in mutated domestic forms of fowl.

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Correspondents alone are responsible for statements and opinions expressed. Letters are dated when received in the editorial office.

SECONDARY SEX RATIO, BODY WEIGHT AND LENGTH AND MORTALITY RATE AMONG INFANTS

A study of birth weight and mortality rate among infants is of great importance, especially in Egypt where little or no extensive analysis has been carried out along this line. All single babies who were born in one of the maternity hospitals for poor classes in Cairo during the year 1949 were included. The secondary sex ratio was expressed as the number of males per 100 females born. The mortality rate was calculated by taking the percentage of babies who died within a week after birth to the total number of babies born. The data included 1762 births, of whom 930 were boys and 832 were girls.

The secondary sex ratio was found to be 111.8. This comparatively high sex ratio is in agreement with Marshall[1] who reported that poor classes of people have a higher sex ratio than the average. In accordance with Crew[2] sex ratio was lower in the cool months (105.2) than in the warm months of the year (120.8).

For all babies studied, the mean birth weight and the mean body length were 6.8 lbs. and 19.1 inches, respectively. On the average, boys weighed 0.2 lb. more than girls at birth, thus agreeing with Donald.[3] The babies who died within a week after birth weighed on the average 2.0 lbs. less and were 2.2 inches shorter in body length than those who survived. Summer babies were slightly lighter and shorter than the average.

The mortality rate averaged 12.6 per cent. Undernourishment and premature birth are believed to be the main causes for such a high rate of mortality. Spring had the highest rate of mortality (17.9 per cent), while the fall had the lowest one (8.9 per cent).

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GENE-FREQUENCIES AND MENDELIAN RATIOS

In 1908, Hardy and Weinberg independently stated a basic principle of population genetics which has since been discussed in a number of elementary textbooks of genetics[1, 2, 3, 4, 5] (Sturtevant and Beadle, 1939; Snyder, 1940; Riley, 1948; Stern, 1949; Sinnott, Dunn, and Dobzhansky, 1950), and which has been well explained by Stern.[6]

According to this theory, in a sexually-reproducing population which reproduces by cross-fertilization, the frequency of two alleles will reach an equilibrium in one generation and will tend to remain unchanged from one generation to another so long as there is random mating, no tendency towards a differential survival of the various types and no mutations and provided that the population is a large one and there are no additions to it from the outside once it has arisen. The proportions of the different phenotypes will depend on the relative frequencies of the two alleles and therefore a dominant allele might be present with so low a frequency that recessive phenotypes may considerably outnumber the dominants. It follows, then, that the dominant or recessive nature of a particular allele cannot be determined from the relative frequencies of the various phenotypes. This principle has been recognized for a long time but has not been universally understood nor appreciated, and occasionally an attempt is made to interpret population ratios in terms of a factorial analysis. This procedure is especially tempting if the population ratio happens to simulate a Mendelian or a modified Mendelian ratio.

One of the more recent population studies which have been interpreted in terms of Mendelian ratios is Schütte's[7] (1949) analysis of flower color in *Romulea bulbocodioides*, a South African member of the Iridaceae. In his introduction, Schütte states, "Occasionally colour differences have been used to separate very similar plants into two species differing only in colour. Yet colour differences are often of a purely genetical nature and follow the ordinary Mendelian Laws of Inheritance. Where colouration is due to simple genetical factors, it is sometimes possible to observe this from ordinary field observations." Studying plants on Rondebosch Common in Cape Town, he counted all the plants in a number of areas each a measured square meter starting with random sampling, but later observing only "hybrid areas."

Eleven random samples yielded areas varying from 36 yellow and 31 white to 10 yellow and 55 white with three approaching a ratio of 9 yellow : 7 white. These random areas showed numerous large patches of either yellow- or white-flowered plants and were therefore considered to result largely from inbreeding. Twenty areas which were believed to contain only plants which had arisen by cross-fertilization yielded 392 yellow and 344 non-yellow plants, or 53 per cent yellows; all but four of these were considered as indicating "a dihybrid ratio" (*i.e.*, 9:7) although no statistical constants were given. Schütte suggests that more homozygous types would have been expected and that their infrequency must indicate very intensive cross-pollination.

The interpretation given for these results is that yellow is dominant over non-yellow. The non-yellows include white, cream, and pale-yellow types. It is suggested that the pale yellows and creams are heterozygotes but no frequencies are given for the various non-yellow types in the twenty "hybrid areas."

If the Hardy-Weinberg law is applied, a ratio of 9 yellow:7 non-yellow could be approximated even if yellow is recessive to non-yellow provided that the frequencies of the yellow and non-yellow genes are respectively 0.73 and 0.27. With this gene frequency, the population should consist of 0.53 yellows, the homozygous recessive, 0.39 heterozygotes, and 0.07 homozygous whites. If the last two classes form Schütte's non-yellows, the ratio should be 0.53 yellow:0.47 non-yellow, which is very close to the ratio from his Table 2. In other words, his observed ratios could be obtained even if yellow is recessive to white and they do not prove that yellow is dominant as he assumed. Since he did not tabulate the pale-yellow and white types separately in Table 2, there is no evidence as to how close his ratio comes to the calculated if, as he suggests, the pale yellows are heterozygotes.

Although the Hardy-Weinberg Law may not be strictly applicable to Romulea since there is no evidence that this plant does not reproduce in part by self-fertilization and since there may be some vegetative reproduction from bulbs, the possibility that ratios like Schütte's can be obtained on hypotheses other than his shows that it is dangerous to assume the dominance of a given gene from studies of the frequencies of various phenotypes in a natural population when only two phenotypes can be distinguished.

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THE HOMOLOGY OF THE "NOTOCHORD" FOUND IN PTEROBRANCHS AND ENTEROPNEUSTS

While examining sections of the curious pterobranch *Atubaria heterolopha* Sato, a suspicion occurred to the writer as to the homology of the "stomochord"—the so-called notochord. This structure is a dorsal diverticulum of the foregut, ending near the brain. A similar structure is found in the enteropneusts. Bateson (1884) was the first to homologize this with the notochord of the vertebrates. He based this idea mainly on the fact that it is a dorsal outgrowth of the digestive tract, and the component cells are vacuolated. This view was accepted by most subsequent authors, and gave rise to the name of "Hemichorda" or "Adelochorda" to designate the group to which these animals belong. A few authors, however, disagreed with this view. Spengel (1893), for instance, supposed that the structure in question is merely a pre-oral part of the gut.

To the writer the "stomochord" seems to be rather a homologue of the anterior lobe of the hypophysis in vertebrates, because it is a diverticulum of the anterior end of the digestive tract, and its blind end is closely apposed to the cerebral ganglion in pterobranchs. It may be objected that, while the stomochord is believed to be derived from the endoderm, the anterior lobe of the hypophysis is of ectodermal origin. But, there is a possibility that the stomochord is ectodermal in origin at least partially (cf. Spengel, 1893). This point would merit a crucial reexamination of adequate material.

The notochord in vertebrates, as well as in cephalochords, arises from the dorsal wall of the archenteron throughout nearly its whole length, and becomes independent of the latter in an early stage of development. The notochord in urochords also develops from endodermal cells intimately related to those giving rise to the digestive tract. It is, however, restricted to the posterior region of the body, without any relation to the brain. Thus, the notochord in all these chordates shows a sharp distinction from the stomochord in pterobranchs and enteropneusts.

The subneural gland of urochords has been homologized with the hypophysis. This structure originates as a part of the brain vesicle of the larva, and becomes isolated from the latter, and acquires a duct opening outside or in the pharynx. This mode of origin reminds us of the posterior lobe of the hypophysis in vertebrates. Possibly, the duct is the homologue of the stomochord in pterobranchs and enteropneusts. At any rate, it seems more natural to homologize the stomochord with the hypophysis, particularly its anterior lobe, rather than with the notochord, in vertebrates.

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CONCERNING FLUCTUATIONS IN POPULATIONS OF THE PROLIFIC AND WIDELY DISTRIBUTED MUSKRAT[1]

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Even among rodents, the muskrat, *Ondatra zibethicus* in its 16 described subspecies, is notable for its fecundity; and its importance in the fur trade has focussed a great deal of public attention on its numerical status. Although its annual fluctuations may often show neither the extremes nor the comparative rhythmicity of the fluctuations of some of the allied voles (*Microtus spp.*), the muskrat is a mammal that *can* vary decidedly in abundance from one year to another.

Since Johnson's (1925) work in New York, the muskrat has been investigated under free-living conditions perhaps as much as any subhuman mammal on earth—mostly as a fur-bearer in its native North America and its new range in northern Eurasia (Artimo, 1949), but also as an introduced pest in central and western Europe (Ulbrich, 1930; Storer, 1937; Warwick, 1940). The muskrat's psychological traits have not encouraged extensive laboratory experimentation, but the species living "naturally" has proved to be a suitable vehicle for the exploration of rather broad population phenomena.

Most of my own participation in these investigations may be said to have begun in Iowa in 1934, after an earlier (1915–28) background of experience as a hunter and fur trapper in marsh and stream areas of South Dakota and northern Minnesota.

The principal objective of the Iowa program of muskrat studies always has been to learn more about the rules of order governing the distribution and maintenance of populations in different types of habitat. In connection with the program, I spent approximately 25,000 hours on field research on the muskrat and its associated biota, in addition to supporting work in laboratory and library and on correspondence and manuscripts. "Key" data were sought from definite observational areas, the same places (totalling about 30 square miles) being regularly kept under observation from year to year. Emergencies, epizootics, population adjustments, or other events affecting the local or regional status of the muskrats were followed as closely as time and techniques permitted. The intensive work on observational areas was supplemented by enough travel outside of Iowa to gain some familiarity with representative parts of about four-fifths of the muskrat's geographic range in the United States and Canada. This out-of-state travel was substantially aided by special grants from the Wildlife Management Institute and by the excellent cooperation of the U. S. Fish and Wildlife Service, the Hudson's Bay Company, and the Manitoba Government.

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As yet, comparatively little has been published on our results, except for preliminary treatment of specific topics (see, for examples, Errington, 1941, 1943, 1946). However, the area data in my possession recently have been written up and, together with introductory material and a review of the biogeography of muskrat subspecies, comprise the first three parts of a four-part book manuscript. The fourth part of the book, which is the synthesis, remains to be prepared, but the trends revealed by the data look sufficiently clear to warrant summarizing, especially in so far as they may illustrate differences between the fundamental and the incidental in population dynamics.

PHYSICAL ENVIRONMENT AND MUSKRAT DENSITIES

Apart from a few special divergences in behavior, such as certain western subspecies not building the usual lodges in marshy habitats and the drowning of *O.z. rivalicius* under ice in Louisiana (O'Neil, 1949), muskrats may be expected to respond to opportunities and crises in much the same way over their occupied range in the northern hemisphere. From beaver pools in mountain streams to desert waterholes, glacial marshes to irrigation seepages, river bayous to brooks, ditches, and lakes, the three widely distributed subspecies that I have worked with in detail (*zibethicus, cinnamominus* and *osoyoosensis*) live like muskrats anywhere, whether confronted by subarctic or subtropical extremes.

Within the limits imposed by its adaptations for a semi-aquatic existence, this rodent, with its peculiar combination of frailty and ruggedness, often demonstrates a remarkable ability to stay alive, to live somehow, at least at times, up to the very edges of what may be considered habitable range. Under stimulus of emergencies or population pressures, the species may be forced to overflow the better habitats, even to the extent of making massive, lemming-like pioneering thrusts into totally uninhabitable range.

It should not be quite a truism to state that the number of muskrats to be found in an area *partly* depends upon essential water, food, and shelter, and the relation of these to each other. A muskrat must have more than a will to live to endure nine months of alpine or arctic cold under several feet of ice. The species is responsive to good sources of food—corn fields bordering streams or heavy stands of cattails, bulrushes, and duck potatoes in marshes—as long as its minimal requirements for space and water are met. Water, itself, may mean the difference between muskrats living and muskrats dying, but, in temperate regions, depths of a foot or two that the animals can depend upon are preferable to great quantities that may through sudden fluctuations, wave action, drowning of vegetation, etc., bring about other complications. Shelter may be afforded either by lodges built in marshes or by burrows dug in banks, or, during emergencies, by land retreats. The usually inferior grades of muskrat environment include large rivers or open lakes without much food, or streams or shallow sloughs that go dry except in wet weather. Among the superior places are tile-fed drainage ditches running through cultivated lands and lush marshes having bottoms well covered by fresh or brackish water.

One of the most influential factors in the lives of muskrats now recognizable from the population case histories on record is that of *density*, or the numbers of the animals that are *already* present in a habitat as the annual breeding season gets under way or as population adjustments occur in late summer and fall. Pearl and Parker's (1922) early conclusions as to the importance of the density factor in regulating populations have been substantiated in many ways by the muskrat data.

The functioning of the density factor in muskrat populations may or may not be attended by visible food shortages or by increased fighting or disease losses or by migrations. It may be seen to be both interlinked with and separate from the physical environment. Greater restlessness and a compounding of troubles are typical manifestations to be noted as densities rise past the levels that are well tolerated or that can be well accommodated.

On the whole, the long-term records from observational areas in Iowa and elsewhere reveal strong tendencies for spring-to-fall increases of muskrat populations to conform to mathematical formulas that *differ with the area* but which for a *given area* may remain *apparently unchanged* for years at a stretch.

At one extreme, very low densities may be expected to show low rates of increase between spring and fall. Survival rates of the young born of low populations living in good environment may be high, but irregularities in mating may reduce the number of young born—all of which is quite in keeping with the underpopulation phenomena explored by Allee and coworkers (see Allee, Emerson, Park, Park, and Schmidt, 1949:399–405). Nevertheless, the *lower* of the densities that permit *efficient* mating tend to show the *higher* rates of increase. For a good Iowa marsh having moderately low breeding populations, averages of 12 to 16 or even more young may actually be raised per adult female during a "normal" breeding season. For the same marsh having higher breeding populations, the net productivity per adult female tends to decline, until, at top-heavy densities, the females may successfully rear averages perhaps as low as two or three young, besides being subject to greater mortality themselves.

The inverse relation usually to be seen between population levels of adult muskrats during May and June (the principal breeding months) in Iowa habitats and the rates of population gain by fall is in some ways reminiscent of the upper asymptote of the Verhulst-Pearl-Reed logistic curve, which is so widely applicable to population growth of organisms (Pearl, 1937; Allee *et al.*, 1949:301-315). The two methods of plotting data on coordinate paper overlap only in part. I have used them both whenever possible but have found that, for a species as subject to variable reductions between fall and the next year's breeding season as can be the muskrat in a cold climate, spring-to-fall rates of gain in relation to adult densities have revealed more of analytical value than have year-to-year population changes. Of course, numerical data on adult muskrat populations present during the main breeding season are not the easiest to obtain, whereas the fur trade may give us records on local or regional trapping catches and fall lodge counts usable as indices of the annual changes. We may often have a series of data sufficient to delineate a logistic curve if nothing more.

The lining up of a string of data points along either a curve of inverse gains or a logistic curve implies not only the self-limiting influence of the density factor but also compensating adjustments in rates of gain or loss and a certain stability of what Pearl (1925) called the "absolute base from which the law operates." Were it otherwise, pronounced variations in numbers of young born to a given population or in mortality suffered through specific agencies would show more *net* influence on populations than they commonly do, in that they would be more disruptive of conformation to evident mathematical patterns. In contrast with the Darwinian view of close interrelationships between population changes and what long have been assumed to be limiting factors, populations of muskrats (among other vertebrates) have repeatedly taken many big differences in vital statistics in stride.

The better the success of north-central muskrats in rearing litters born early in the breeding season, the sooner the year's breeding is likely to terminate (as in early or mid-summer, after the birth of one to three litters) and the greater may be the losses suffered by the young that do happen to be born late. Sometimes, the whole late-season (late June to September) production may simply be frittered away if most of the early-born young have been reared. Conversely, the poorer the success in rearing early-born young, the greater the likelihood of breeding being prolonged in compensation (as into August and September) and of unusually large proportions of late-born young surviving. This has been especially well studied in stream habitats in which nearly all of the young born up to mid-June were drowned in floods.

The extent and effectiveness of late breeding is plainly conditioned by the psychology of the muskrats. Diminishing of population tensions affords one of the best stimuli for reproduction. Where the animals live in superb but underpopulated habitats, we may get averages approaching four litters born per breeding female between April and August, with up to three-quarters or more of the young (late-born as well as early-born) being reared. Under such conditions, the late breeding is stimulated not by death of the early-born but by the early-born being able to spread safely into places away from those frequented by the breeding adults.

Other evidences of automatic adjustments may be seen as different types of losses substitute for one another without perceptibly affecting the over-all patterns. When, for instance, mink predation upon insecure young muskrats was severe, losses from other agencies (including other species of predators) tended to diminish; when mink predation was light or lacking, losses from other agencies tended to mount up and to do the eliminating of biological surpluses instead. Yet, when the muskrats introduced in the British Isles were successfully extirpated through deliberate campaigning (Warwick, 1940), we had an example of specialized predation that did not stop with elimination of a surplus. Past a certain point, the substitution phenomena in losses due to human and "natural" agencies and the stimulation of breeding through decreased population pressures no longer operated in effective equilibrium. Although ordinary fur-trapping or control-campaigning (as in central Europe) may cut muskrat populations down to levels below those expected to result "naturally" and thus be rated as having truly depressive influence, moderate intensities of human exploitation or persecution have their compensatory aspects. In other words, a great deal of the direct pressure that man applies to the species merely takes the place of "natural" agencies in the elimination of biological surpluses.

An abundant vertebrate and one occupying such a diversity of habitats and such an immense geographic range as the muskrat may present so many special cases as to make generalizing hazardous. It should still be proper to discuss the tendencies toward virtually endless counterbalancing between rates of gain or loss that may be expected to occur within the mathematical formula set for an area by the densities and psychology of the muskrats themselves.

The capacity for accommodation of environment for muskrats is hard to define, under that name or "threshold of security" or "biological base" or some other. It may show a noteworthy resistance to change, irrespective of many ordinary seasonal or year-to-year variations in water, food, and weather, but environmental changes may be of sufficient magnitude to affect a muskrat population.

Cattails or similarly favored foods may be killed by flooding, plant diseases, insects, livestock pasturing, or overutilization by the muskrats. Or, a muskrat marsh may deteriorate in time from no demonstrated cause, or perhaps as food plants fade out with ecological succession. Or, a food-poor body of water may dry up under exactly the right conditions to promote growth of cattail or bulrush seedlings, later to be partly reflooded and to turn, accordingly, into a splendid muskrat marsh in a single growing season. Events of this sort may decidedly change the status of the local muskrats, often with comparative suddenness.

There is plenty in both the resiliences and rigidities underlying population patterns that cannot be explained on the basis of our present knowledge. A dominant self-regulating mechanism may be identified with the territorial or spatial intolerances displayed at least during breeding seasons if not at other times by very many populations of vertebrates (Nice, 1941). Among the markedly territorial of the vertebrates, we may detect a certain awareness of when "enough is enough," and, as social relations tighten, the individuals or groups possessing the equivalents of established property rights have a competitive advantage. Following the breeding months, social relations usually relax, and we may have observable phenomena (including loss rates) that look as if they might be considerably less a function of territoriality, *per se*, and more a function of the disadvantages of immaturity and of exposure to the dangers of unfamiliar places, to chance encounters with predators, to sources of infectious diseases, to climatic emergencies, accidents, etc.

One of the density phenomena that I have had trouble trying to make sense out of is the way in which big environmental and population changes have occurred in constituent parts of a large area without noticeably affecting the biological base holding for the area's muskrats as a whole.

In central Iowa, I had noted that local differences in numbers and productivity and in post-breeding movements might occur from year to year, to be offset by compensating differences elsewhere in the neighborhood. Some places looked crowded while others looked underpopulated, though the population of the observational area, considered as a unit, continued to conform to a year-to-year pattern.

Counterbalancing adjustments are particularly well indicated by records furnished me by the Hudson's Bay Company and the Manitoba Government. For an area of nearly three-quarters of a million acres near Cumberland House, Saskatchewan, the annual muskrat catches, plotted against time, 1930–35, defined the slope and both asymptotes of a logistic-type curve; then, following an engineering program to improve water levels for muskrats over a large part of this area, the 1939–46 catches again showed a tendency to line up along a logistic curve, but a curve having a distinctly higher base than that operative earlier. This much would seem to be a result of adding a large amount of muskrat habitat to what had been in the area during the preceding decade, but further engineering improvements in 1946 increased by about a third the acreage of productive marshland under management without appearing to have raised, up to 1951, the base governing the configuration of the logistic curve for the area's muskrats. Moreover, scrutiny of the details recorded for the constituent parts of the managed area left me with an impression of about every conceivable local variation in the year-to-year fortunes of the muskrats and the status of their habitats. Despite the local variations and the interplays of complexities, what looked like an over-all pattern somehow was maintained for years at a time, and similar trends are so well illustrated by the data from other Canadian areas that we cannot take for granted that they just happened.

DEPARTURES FROM "NORMAL" PATTERNS OF POPULATION ADJUSTMENTS IN MUSKRATS

For all of the tendency of a muskrat population to increase or to suffer losses according to a scale of rates that might be called predetermined by the density factor in relation to a given habitat, failures to conform are quite to be expected. Many of such failures are only temporary and readily understandable, as when droughts or epizootics may kill practically entire populations.

It should not be assumed that any drought or any die-off from disease necessarily has real depressive influence on the muskrat populations affected.

Droughts may not be of extreme deadliness to muskrats as long as the animals are neither deprived of the necessities of life nor forced into unsafe living routines. In the warmer months, muskrats generally withstand drought, even carrying on productive breeding, as long as water remains in their burrow entrances. Drought losses (or indirect losses associated with drought) occurring at times of year when biological surpluses are being eliminated in one way or another may be largely compensated as by decreased losses through other agencies, if the droughts are relieved before they progress too far.

Like compensations may attend losses through floods, violent storms, or epizootics, if these are not overwhelming; if light, the losses may be absorbed in the population adjustments; if heavy, the losses may or may not count in depressing a population, depending upon time and circumstances.

Should any of the numerous types of emergency or disease losses depopulate only parts of an area of muskrats prior to the end of a breeding season, overflow of adjusting animals from unaffected parts may soon refill the vacant quarters (always granting that conditions there again become favorable), sometimes so completely in a few weeks that drastic losses may have little or no net effect.

The distinction to be kept in mind in appraisals of the significance of emergencies and epizootics is whether the losses therefrom really reduced an otherwise secure population so that it remained below a level that it would have maintained or whether the losses merely gave otherwise doomed animals a chance to live that they would not have had.

Severe losses may also be compensated biologically after considerable delay. Efficient fur harvests removing half to four-fifths of muskrat populations between fall and spring surely may be said to depress the populations but they may also greatly relieve the tensions of the coming breeding season and thus promote more rapid recovery. Not only may the deadliness of fur harvests be partly or wholly offset through lessening the toll from fighting, subhuman predation, wandering, etc., and through stimulated reproduction, but it may also help to prevent epizootics from gaining headway and the more crowded of habitats from being destroyed by overuse.

But cataclysmic reductions of muskrats can occur, and unforeseeable depopulation through drought or disease on a county-wide or larger scale may understandably upset calculations from an area's density figures and recorded history. Either drought or disease may dominate for long periods the whole life equation of muskrats, either locally or regionally.

The droughts of the thirties left muskrats alive only in the most exceptional habitats in the Dakotas, eastern Montana, and the southern Prairie Provinces, beside reducing the species over a much wider range during the driest of the dry years. During droughts, muskrats may die outright from thirst or heat, while wandering cross-country or while staying tenaciously in their home ranges. Spectacular losses may occur through the medium of subhuman predation, as mink, foxes, coyotes, dogs, raccoons, large raptorial birds, and other opportunistic flesh-eaters respond to the unusual availability of acceptable prey.

Drought also promotes trouble for the muskrats by concentrating them in the better habitats, to the accompaniment of increased fighting and restlessness (hence, increased vulnerability to enemies, as well) and miscellaneous complications; it may facilitate spread of infectious disease, as water may become foul or as sick animals may wander from puddle to puddle along a drying marsh bottom or stream bed; it may indirectly result in winter-killing, as shallow waters freeze to the bottom and seal off the food that would ordinarily be available in unfrozen water or mud. In the same way, a snowless winter in northern regions may allow very deep freezing of marshes. I have observed heavy mortality of muskrats under such conditions in Iowa and South Dakota, and Canadian correspondents wrote me that, due to lack of snow in the winter of 1949-50, the frost line sank five feet in northern Manitoba and Saskatchewan, all but wiping out the muskrats over a vast region of marshes and shallow lakes.

Since 1943, we have carried on in Iowa intensive studies of the epizootiology of a muskrat disease, of undetermined etiology but known over much of North America from its hemorrhagic and necrotic lesions and what can be great deadliness. Epizootics of this hemorrhagic disease (or of tularemia and conceivably of other virulent infections, at times) may make extensive areas nearly uninhabitable for muskrats for years, and so exert a population effect that is real, indeed.

The hemorrhagic disease may introduce highly confusing variables into population analyses, apart from questions as to numbers of muskrats killed thereby and the extent to which losses were or were not compensated. Sickening animals may have propensities for overland movements, for coming out on the surface of the ice in winter, for fighting among themselves, for being killed by predators, or for doing something else that could suggest things other than disease in the population picture. Undetected epizootics in muskrats—and clear evidences of disease mortality may easily be missed unless an observer is working in the right place at the right time and knows what to look for—have been wonderfully fruitful of public misappraisals of activities of predators.

Let us consider a rather common winter situation on a north-central marsh on which minks are present and muskrats are favorably situated with respect to food, shelter, water, and their own population densities.

We may see that minks repeatedly invade muskrat lodges and sub-surface retreats and that this may go on for weeks or months without having any lethal significance to the muskrats, whatever. The minks do show a preference for muskrat flesh and are usually able to kill muskrats if any subhuman predator can; but functional grown muskrats having environmental and psychological odds in their favor rarely need to let themselves be killed by minks. Particular lodges may be "bored" by minks a half dozen times or so in a winter, to be repaired or not repaired as the muskrats may choose; to be repaired immediately after the minks leave or weeks afterward, frozen plunge holes notwithstanding.

Habitual intrusions into muskrat retreats may reward the minks in other ways than by improving their chances for eating muskrat, but this will not be discussed here. Be it understood that a die-off from hemorrhagic disease may leave up to a dozen or more dead muskrats in a lodge and in northern latitudes these dead may remain refrigerated well into the spring. Responsiveness of minks to these "windfalls" of a favorite food may be manifested by unrepaired mink holes in lodges and burrows, latrines of mink scats made up of muskrat remains, of dead muskrats being dragged over snow or ice or shore, and heads, feet, tails, large bones, blood, and patches of muskrat fur scattered about. It is nothing exceptional to find minks inside of lodges cut open for examination, along with partly eaten muskrat carcasses. Intact carcasses may be strewn or lying in piles about the chambers, much as when muskrats actually are being killed in large numbers by minks during freeze-outs or drought crises.

Outdoors men having conventional ideas of the nature of mink predation upon muskrats and lacking knowledge of the workings of the hemorrhagic disease look at such scenes with conviction that the minks had been slaughtering. In my routine examinations of the viscera of muskrats retrieved from winter mink retreats during times of no evident emergencies, I have practically always found diagnostic lesions in muskrats found dead in groups and in a large proportion (though not all) of those found singly away from known foci of infection. Keeping informed as to current food habits of the minks has proved to be most helpful in discovering "hotspots" of the hemorrhagic disease and in tracing their spread, especially while the marshes are frozen. And, after the ice disappears in the spring, it is often possible to date the winter's dying for different sites from the sexual advancement shown by the more intact and better preserved of the victims.

Departures from "normal" patterns of population adjustments may also be expected in consequence of the "cyclic" or periodic influences next to be taken up.

ON THE EXPLORATION OF "CYCLIC" PHASES IN MUSKRAT POPULATIONS

Recent reviews by Elton (1942), Dymond (1947), Siivonen (1948), Kalela (1948, 1949), Franz (1950), and Christian (1950) illustrate the scope of modern investigations of cycle-like fluctuations in vertebrates. The literature on the subject is tremendous, and important contributions are scattered through everything from sporting magazines to museum reports. Attention has been paid to "cyclic" and related phenomena principally in the northern parts of the northern hemisphere but not exclusively there, as may be seen from the work of Bodenheimer (1949) in the Middle East and of Wodzicki (1950: 139–141) in New Zealand.

Most of the seemingly periodic fluctuations in year-to-year populations of mammals and birds occur with something near either a three-to-four year rhythm or a 10-year rhythm. These two cycles have been considered by Vinogradov (1934) and by Siivonen (1948), in particular, as being related, but a great amount of the published evidence is still non-committal. Regional differences, species differences, and miscellaneous irregularities in fluctuations have beset studies of cyclic phenomena so much that some investigators have disclaimed belief in any periodicity at all while others have concluded that each species has its own local cycle.

As I see it, the outstanding source of confusion among reputable students of population cycles is overemphasis of fluctuations of animals or use of such fluctuations as the sole criteria of cyclic behavior. Variations in phases of ups and downs of populations may truly bewilder anyone looking for synchronies, and the exceptions are at best a hindrance to easy generalizing. I would state, furthermore, that fluctuations in numbers of animals from one year to another can be very misleading. Unfortunately, fluctuations furnish for many species about the only data we may have to go on.

It is a fair question to ask how a species may logically be classed as "cyclic" if it doesn't show periodic fluctuations. If the fluctuations aren't periodic, then just what do we look for?

We must look for common denominators, for synchronies (or lack thereof) in life processes over a sufficiently long span of years to bring them out if they are there.

We must also keep in mind that the numbers of animals comprising a population are resultants of a multiplicity of factors. Important in the regulation of numbers of a wide variety of vertebrates may be intra- and interspecific competition or the density factor, plant succession, human land use, climate, lethal emergencies, epizootic disease, and uncompensated predation, including that frequently taking the form of hunting pressure by man. In addition to these, some investigators, myself among them, think that a probable extramundane factor may affect living things in an essentially periodic manner. It is clear that the population impacts of at least the better defined of these factors vary with species, time, and place.

To the muskrat, a series of drought years, whenever it comes, may have greater population effect than anything I have ever observed to synchronize with any apparent "cyclic" phase. On the other hand, while Grange (1949) has convincingly emphasized the importance of forest growths following fires to the population status of the snowshoe hare in North America, my feeling is that something still unknown may be more of a master factor behind the "classically cyclic" fluctuations of the species.

In developing this thought, let us restrict our attention to the central and eastern regions of the continent, chiefly because of my greater familiarity with the fauna of these regions and the population researches that have been carried on from Iowa, the Dakotas, and eastern Saskatchewan east to the Atlantic. During the past three decades, this geographical expanse has yielded some of the best long-term data we have on wild vertebrates.

For the times for which comparable data exist, the native grouse of the above regions fluctuated about as the snowshoe hares did, and, in the agricultural lands of north-central and northeastern United States, the fluctuations of the introduced ring-necked pheasant were in fair synchrony with those of the hares and grouse. The hares, grouse, and pheasants rather generally reached maximum levels in or about the years ending in ones and twos and minimal levels in or about the years ending in sixes or sevens. At the risk of oversimplification, it might be suggested that the hares, grouse, and pheasants may be more susceptible to the so-called "cyclic influence" than the other resident species of small to medium sized mammals and birds, which sometimes did and often did not fluctuate synchronously. Among the latter species we have the muskrat.

The muskrat has been classed as non-cyclic, as having a cycle inverse to that of the hares and grouse, as having a cycle running parallel to that of the "classically cyclic" species though preceding it in phase, and as fluctuating according to the usual "10-year game cycle." Elton and Nicholson (1942) ascribed—I think rightly—to long-term fluctuations in rainfall a great deal of the annual fluctuations in trappers' catches of Canadian muskrats between about 1850 and 1940.

Let us use the "key" years of the fluctuations of the hares, grouse, and pheasants somewhat as sighting points and see what appears to synchronize on the muskrat areas kept under observation.

The "cyclic low" of 1936–37 coincided with a terrific drought over most of my study areas. However, local muskrat populations escaping the consequences of drought showed depressed rates of gain and increased rates of loss in relation to their densities and the quality of habitat available to them—as also did bobwhite quail and a number of other species that were intensively investigated at this time (Errington, 1946).

The last "cyclic low," 1946–47, was not accompanied by any extremes of drought or other unfavorable weather conditions over my study areas, and the central Iowa muskrat populations that were most closely observed maintained themselves at moderate to fairly high densities. In fact, central Iowa had more muskrats during this "cyclic low" than during 1941–42, two years assigned on other grounds to the period of the "cyclic high"! Over thousands of square miles of muskrat range to the west and north of Iowa, populations of the species did decline in broad chronological agreement with population declines of the hares, grouse, and pheasants.

But the central Iowa muskrats, despite the lack of agreement between their fluctuations and those of the "classically cyclic" species, showed plenty of other evidence of being affected by a common depressive agency.

Data were obtained on the sizes of nearly 1,600 litters conceived by fully adult muskrats on the Iowa study areas. The averages were 6.6 for 78 litters in 1935; 6.3 for 43 litters, 1936; 6.5 for 41 litters, 1937-38; 7.2 for 68 litters, 1939-40; 8.3 for 79 litters, 1941-42; 7.5 for 300 litters, 1943; 7.0 for 362 litters, 1944; 6.9 for 108 litters, 1945; 6.4 for 65 litters, 1946; 7.2 for 118 litters, 1947-48; 8.1 for 83 litters, 1949; 8.0 for 211 litters, 1950. Most of the litters recorded for 1943 and 1944 were conceived by members of very top-heavy local populations, hence subject to density depression to a greater extent than were the sizes of litters conceived during the other years, for which densities of observed breeding populations were more nearly comparable. Even so, there is no reason to think that the average sizes of the 1943-44 litters were lowered by more than about a half muskrat because of unusual density effects, and, with these density effects dissociated, it may be judged that the annual differences in average litter sizes reflect something else.

The significance that I see in the changes in average litter sizes of the Iowa muskrats is the lining up of their years of minimum, intermediate, and maximum values with those of the corresponding changes in numbers of the region's hares, grouse, and pheasants. Actually, the changes in litter averages appeared not to affect the population levels of the muskrats, but they are of exceptional interest as a phenomenon, implying as they do an agency exerting some sort of depressive influence upon the physiology of the muskrats as well as upon the evidently more sensitive hares, grouse, and pheasants.

Relaxation of this depressive influence—whatever the latter may be—with the approach of the period of the "cyclic high" is suggested by data on the sexual status of young female muskrats trapped in the fall and early winter of the calendar years of their birth. Of 261 specimens of females of the year examined in November and December, 1936-38, all were sexually immature, as were all of 312 examined, 1939-40. Most of the specimens for 1936-38 were taken from places having late-season conditions about as favorable for late breeding as they usually are, but this was not true for the dry late summers of 1939 and 1940. For 1941-43, 788 females of the year were recorded as being sexually immature by early winter, but three specimens originally classed as small adults that had conceived single late litters were later thought to have been young breeding precociously; it is probable that more precocious breeding might have been shown by the samples for these years had the majority of specimens not been taken from populations of sufficiently high densities to discourage late breeding, generally, whether by full adults or by precocious young. For 1944-46, years accompanied by some to a great deal of late-season breeding on the part of bona fide adults, 1,028 females of the year showed no sign of sexual precocity. For 1948-50, of 702 females classed as young of the year that were examined among the trappers' carcasses, 24 had conceived a late litter in the year of their birth, and the preponderance of this precocious breeding occurred in 1950, coincident with what appears to be the beginning of a new "cyclic high."

The precocious breeding so far recorded has had trivial effect on the population status of Iowa muskrats, and I should not expect it to be important for the species at any latitudes at which late-born young do not have time to get a good start before cold weather. Like other reproductive changes accompanying changes in "cyclic" phases, such unusual precocity may give us a lead as to possible common denominators.

Returning again to sight along the "key" years of the hare, grouse, and pheasant fluctuations, we find the detailed data from the muskrat studies still more informative.

During the one "cyclic high" of 1941-43 that has been studied thoroughly on the Iowa areas, the muskrats occupying first-class marshy habitats were observed to tolerate maximum breeding densities up to the equivalents of nearly eight pairs per acre over sizable tracts, whereas, during the "cyclic lows" of 1936-37 and 1946-47, the breeding densities in similar habitats levelled off at around two or three pairs per acre, even when large numbers of homeless individuals were frequenting the peripheries. Both at favorable and unfavorable "cyclic" phases, the maximum breeding densities were patently determined by what the established residents would put up with, the savageness of the fighting and other manifestations of intolerance being similar to view for eight-pair densities in 1941-43 as for three-pair densities in 1936-37 and 1946-47.

These differences in tolerance are amply illustrated by the data on annual distribution of breeding territories of muskrats on the Iowa study areas. The spacing of territories with respect to each other showed a remarkable uniformity during the years of "cyclic lows"; in typical marshes and streams, they were established and maintained in attractive and rather unattractive parts alike; and, if the animals were motivated in their selection of territorial sites by anything beyond passable living requirements, it looked like a desire to get well away from neighboring territories.

Territories of other years, that is, all years falling in chronology between the "cyclic lows," not merely during the "cyclic highs," were spaced much more with reference to differences in quality of habitat. There might then be massing of three or four territories along a streamside corn field or in a clump of cattails in one corner of a marsh. Social intolerances may be witnessed during these local territorial concentrations, too, but the animals act as if disposed to adjust to considerable crowding in response to superior grades of habitat. At very high densities—say, equivalents of around a dozen breeding pairs settled per linear mile of a small stream—territories may be spread through the poorer as well as the better parts; but, at lower densities, the less inviting places tend to be left muskrat-vacant.

Population tensions in the muskrat are naturally accentuated during the main breeding season (April to July, in Iowa), following which is a period of minimal friction, continuing about up to the coming of hard frosts. But, post-breeding adjustments on the Iowa areas have not been any more exempt from apparent "cyclic" influence than have the adjustments of the breeding season.

Although muskrats in late summer and early fall of any year may engage in local movements as food or water conditions deteriorate or as wintering quarters may be sought, such movements normally take place as orderly extensions of home ranges or along fairly definite avenues of travel. Except at times of great crises and "cyclic lows," these movements have been almost characterized by participating individuals acting in ways that could be called deliberate, purposeful, and intelligent—that is, for muskrats. During the two "cyclic lows" that we have studied, however, large numbers of muskrats have wandered over the countryside, footloose and desperate and vulnerable to all manner of dangers. In the fall of 1946, they engaged in exceptional wandering in central Iowa for no visible cause; moderate populations seemed comfortably situated in most stream habitats, yet the cross-country movement resulted in conspicuous mortality from motor traffic and put hundreds of strange muskrats into isolated bodies of water.

The excessive restlessness observed for Iowa muskrats during the "low" phases of the "10-year game cycle," together with other evidence, invites the thought that the species behaved at low to moderate densities during the "cyclic lows" in ways similar to those in which it behaved at very high densities during the more favorable phases. It may be presumed that physiological as well as psychological changes were involved and that the psychological changes were conditioned by the physiological.

Further inklings as to physiological changes accompanying changes in "cyclic" phases may possibly be afforded by observations as to times of dominance of the principal syndromes of the hemorrhagic disease.

The typical syndrome has been one of light to moderate intestinal hemorrhages, variable amounts of hemorrhage elsewhere in the viscera, as in lungs and kidneys, and a moderate to heavy sprinkling of necrotic foci over the liver. The interval between exposure and death for this syndrome has been slightly over a week, as shown both by the best field data and by laboratory experiments. This syndrome prevailed from the beginning of our intensive disease studies in the fall of 1943 through the spring of 1946.

From the fall of 1946 to about half way through the spring of 1947 and again in the spring of 1948, another syndrome prevailed, this one manifested by few or no liver lesions and by severe hemorrhages in lungs or intestines. We were unable to determine with accuracy how soon after exposure victims could be expected to die, but this syndrome could be seen to act rapidly, probably killing the animals in a few days and before many liver lesions had a chance to form. Many victims had the appearance of having bled to death from their hemorrhages in lungs, cecum, or rectum.

Between 1943 and 1948, the very young animals dying in the course of sweeping epizootics and picked up along with hundreds of larger victims rarely showed macroscopic lesions.

Epizootics continued, almost annihilating parts of populations that happened to be in line of spread, until early summer, 1950; but the prevailing syndrome had switched back to something like the typical one by the fall of 1948. By the fall of 1950, the disease had almost become nonlethal, manifested by liver lesions (even in the very young) and absence of hemorrhages.

By the fall of 1950, about the only muskrats still dying from the disease on the Iowa areas were at the sites of severe mortality of past years, hence thought to be individuals especially exposed to massive infection. The incidence of recognized sublethal infections in the general populations of central Iowa was also high by the fall of 1950. Of 446 muskrats trapped at large over one of our main areas in late November, 1950, 6.7 per cent were recorded as having liver lesions, as were 13.6 per cent of a lot of 59 trapped from the vicinity of an old "hotspot" of the disease at another place. Few muskrats active enough to be caught in traps showed lesions of the disease in years of great mortality and then usually very slight ones, such as a spot or two on the liver.

In considering our data on the epizootiology of the hemorrhagic disease on the Iowa muskrat areas, I would attribute the observed changes in syndromes to changes in resistance of the host animals rather than to changes in virulence of the infectious agent itself. Between 1943 and the middle of 1950, it did look as if infected population groups quite typically demonstrated their infection by dying. Some evidence of individual immunity was seen in both field and laboratory, but, as a rule, the spread of epizootics could be traced on a given marsh by the nearly complete annihilation of muskrats in disease-swept tracts. Twenty or more dead per acre were retrieved from places having hardly any left alive. Then, by the fall of 1950, the disease was as prevalent over the study areas as we have ever known it to be, yet almost nothing was dying from it.

Without implying that there might not have been changes in the immunity of our Iowa muskrats unrelated to the "10-year game cycle," it may be pointed out that the changes from moderate to severe to moderate again and then to benign syndromes of the hemorrhagic disease so far have appeared to be in chronological agreement with the changes in phase of the hare, grouse, and pheasant fluctuations of the north-central region.

My concept of an unknown factor underlying the apparent synchrony of "cyclic" manifestations is of something depressing certain life processes. Apparently, it may be far more nearly a master factor in determining abundance of some species than of others, but, on the basis of the evidence in sight, I would suspect that animal life generally may be subject to its influence, even if not to the extent of appreciably fluctuating in numbers. Years ago, in my gropings concerning possible mechanisms, I judged that essentially all the manifestations I had been classing as "cyclic" *could* reflect overstimulation followed by exhaustion.

Christian (1950) proposed that "cyclic" declines in mammals may have been due to exhaustion "of the adreno-pituitary system subsequent to the stresses inherent in a high population level, severe climatic conditions, and the demands of the spring breeding season." He reviewed Selye's adaptation syndrome in this connection (see Selye, 1949: 837–867 and earlier writings). Christian also suggested in summary that the "length of the cycle is probably a function of the reproductive potential of the species involved, and this potential is applicable to that portion of the population in excess of the death rate for the development of peaks. The terminating factor is the attainment of a population above the carrying capacity of the environment," also the possibility "that longer daylight, decreased light intensity, and lower temperatures during the breeding season in the north maintain a higher reproductive potential in turn leading to more pronounced cyclic changes than in more temperate climates...."

I do not doubt the validity of the stresses discussed by Christian, but neither his nor the other writings of which I know that are preoccupied with fluctuations of the animals deal with the synchronies in manifestations of periodicities that seemingly may occur at times of no pronounced fluctuations in numbers.

My interpretation of the evidence from the muskrats is that stress has repeatedly accompanied both the attainment of top-heavy populations whenever occurring and the onset of "low cycle" years, whether the species were particularly abundant or not. There still appears to be something about the chronological "cyclic low," that is, the years of late decades ending in sixes and/or sevens in the north-central region, to cause moderate or even low populations to display the symptoms that we associate with overpopulation.

Despite the philosophical hazards and the lack of satisfaction of attempting to explain one unknown in terms of another, I do not see how we may, with scientific propriety, ignore the synchronies of symptoms that have gradually emerged from detailed population data.

Solar phenomena have not been neglected by investigators seeking correlations between periodic fluctuations of animals and possible extramundane influences. There would seem to be a connection between sunspot and weather cycles and so, indirectly, between sunspots and numbers of some kinds of animals. This notwithstanding, the sunspot cycles line up only part of the time with a particular "cyclic" phase shown by "classically cyclic" animal life. As MacLulich (1937) demonstrated for the Canadian snowshoe hares, sunspot cycles passed in and out of phase with the hare cycles if a sufficiently long span of years be considered: "Between 1856 and 1937 there have been eight rabbit cycles, but only seven sunspot cycles; the average length of the rabbit cycle was 9.7 years, but the sunspot cycle averaged 11.0 years."

A fair agreement between the low years of the "10-year game cycle" in the north-central region and the sunspot maxima may be seen for about the first half of the Twentieth Century (Stetson, 1947 p. 148). Alignments between the "high cycle" years for game and the sunspot minima look a good deal less indicative to me, notably in so far as some of the distinctly favorable "high cycle" years may be years of moderately abundant sunspots and as the beginning of "cyclic lows" may be years of no greater (or even fewer) sunspot numbers than were recorded for the "cyclic highs."

I do not know how to evaluate the possibility that the more recent data on sunspots as well as on populations may be the more reliable and that alignment of "cyclic lows" with sunspot maxima in recent decades may for this reason be more meaningful than non-alignment farther back. Without venturing farther outside of my own field of competence than I have any intention of going, I may say that I am unconvinced that sunspot cycles provide the answer for the particular 10-year periodicity that I think I see. At the same time, I do not wish to rule the sunspots out of the equation if they are really in it, though operating in ways beyond my knowledge.

DISCUSSION AND CONCLUSION: INTERCOMPENSATORY TRENDS AND BIOLOGICAL BALANCE SHEETS

Because of the prominence of automatic adjustments shown by the population data with which I have worked, as well as the prevalence of intercompensatory tendencies in the animal kingdom (Solomon, 1949), I feel most wary of computations undertaken to establish definite values in vital statistics of animals. This does not mean that I would discourage mathematical approaches to population problems, for I believe that advances may be made through them if undue assumptions be avoided. Neither does it mean that I am offering a blanket challenge to Chapman's (1931) "biotic potential versus environmental resistance" nor to Deevey's (1947) introduction to life tables for natural populations nor to any other scholarly work that has as its aim a more accurate numerical expression of biological phenomena.

My hope, in this writing, is to supplement the endeavors of biometricians by bringing out some of the things that must be taken into account in arriving at true-to-life formulas.

I question that the biotic potential of my Iowa muskrats could be expressed numerically with even a dependable prospect of 50 per cent accuracy. Not only may the average sizes of their litters vary with "cyclic" phases and intraspecific density (and other factors considered of much less general importance), but the numbers of litters born per adult female in a breeding season also depend upon degrees of crowding and the properties of the habitat. A population of breeding females may conceive or give birth to twice as many young as may another population of like densities and environmental status if the latter population merely happened to have been more fortunate in rearing its early-born litters. One may be reminded of the prompt renesting of many species of birds following destruction or abandonment of early clutches of eggs (Laven, 1940; Errington, 1942).

In another part of a balance sheet equation, we have losses from a variety of factors. Some are density-dependent and some are not; some are intercompensatory and some are not; but a substantial proportion in the muskrat and all other vertebrates with which I have worked personally are both density-dependent and intercompensatory. A usually fallacious procedure is to assign values (arbitrarily or on the basis of research findings) to the different agencies of loss, to add up the values, and to consider the total arrived at as a figure reflecting the workings of Nature. Through this kind of calculating, bad distortions may be dignified by a semblance of scientific methodology that might deceive anyone not familiar with its analytical pitfalls. Unless they consider the automatic shifts taking place within life equations, well may anyone wonder that many thriving species can exist at all in the face of the enemies they have or the losses they suffer!

First, we must not forget that extraordinary mortality may offset itself by *stimulating* reproduction, more young being produced as a result of more dying. Likewise, that the total amount of loss suffered by a given population in a given time and place depends *in part* upon whether or not more individuals have been produced than the available habitat can securely accommodate.

Excess populations for an area may be numerically large or numerically small; they may comprise a large or a small proportion of the total number of individuals present. Sometimes, there may be no real excess population for an area, even for an abundant species. In marginal habitats, there may be no real security for a particular species, even when it may be barely represented. The "normal" range of variations in this respect is such as to warn us against improper assumptions of concreteness. A given muskrat population may appear to be remarkably secure at an average density of 15 per acre; another population may attain that level but be so top-heavy that three-quarters are candidates for elimination in one way or another; another may not be able to maintain itself at 15 per square mile, though temporary densities greatly exceeding this figure may occur.

The case histories of muskrat populations bespeak much regulation by formula, not just a formula in which values for the terms conveniently "stay put" so much as one operating along a sliding scale as populations rise or decline within the framework of toleration limits and environmental requirements of the species, "cyclic" phases, etc. Except in the sense that everything living must ultimately die, life equations of the muskrats tend to be replete with flexibilities.

A value assigned to a mortality factor that merely substitutes for another should not be considered the equal in population significance with one that genuinely brings about a net lowering of the population. What difference does it make biologically if a mink or a fox or another muskrat killed a young muskrat as long as death of that individual essentially made it possible for another young muskrat to continue living? The population impacts of a dominating emergency or epizootic are in an entirely different category. It is in mishandling of losses designated for specific agencies that one is apt to go farthest astray in balance sheet computations.

We are still in explorative stages so far as concern natural checks and balances in animal populations, and the muskrat data give us scarcely more than indications as to what may be fundamental mechanisms. We can see that widely-held concepts as to limiting factors need a good deal of revision. The modern biometrician must be careful not to confuse with limiting factors those population by-products that occur incidental to, but with slight if any influence upon, the population status of a species. He must be careful not to confuse the *fact* of losses with the *effect* of losses.

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A GENERAL MECHANISM OF DIFFERENTIATION BASED ON MORPHOGENETIC STUDIES IN CILIATES

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INTRODUCTION

In the absence of conclusive evidence to the contrary, the concept currently held most widely is that somatic nuclei are equivalent genetically. To account for cellular differentiation on this basis, it is generally assumed that the cytoplasm is endowed with potential morphogenetic plasticity; that different local field conditions canalize given cells into different morphogenetic pathways; that the canalization process is self-reinforcing and entails a narrowing down of developmental potencies; and that genes are or are not effective explicitly, depending on specific substrate characteristics of given cytoplasm. In other theoretical formulations, developed particularly during the last decade, the genome is also assumed to be identical in all somatic cells, but the plastic properties of the cytoplasm are attributed in part to plasmagenes, variously defined.

Yet it has been clearly recognized by embryologists and geneticists alike, that progressive regional diversification of the genome might well occur. Possibly, such diversification could represent an important phase of differentiation. If so, mutational changes could probably not be involved, since normal, orderly sequences of development appear to require a less fortuitous foundation. But metabolic changes of the gene, possibly elicited by specific metabolic changes in the cytoplasm, admittedly could not be ruled out.

Recent studies on ciliates provide initial evidence for such nuclear changes, regionally correlated to differentiative changes in the cytoplasm. The experimental data are of sufficient scope, and their implications of sufficient significance, to warrant a first attempt at integrative interpretation. As presented in the following account, a general concept of differentiation emerges which is consistent with the conclusions of experimental embryology and physiological genetics, and has tentative applicability both to protozoa and to multicellular organisms.

SCOPE OF CILIATE MORPHOGENESIS

For descriptive purposes in the present context, it will often be convenient to distinguish between "morphogenesis," the new-formation of a particular morphological pattern, and "morphostasis," the steady state condition which maintains a particular pattern. Morphogenesis may either precede or succeed morphostasis; both involve differentiation. In ciliates, both processes culminate in the visible expression of surface potentialities: it is primarily the ectoplasmic cortex which endows the infusorian with a particular diagnostic morphology. The principal morphogenetic events are regeneration and fission. Other developmental phenomena occur, *e.g.*, excystment, or unique structural transformations in a variety of parasitic forms. To a large extent, these entail differentiative sequences analogous in part to those of regeneration, in part to those of fission. In general, sexual processes *per se* have no immediate morphogenetic aspects.

Regeneration, elicited by structural or physiological injury, involves either direct replacement of injured or lost parts, or resorption of the uninjured parts and redifferentiation of an entirely new set of organelles. In fission, either a second set of organelles is developed in addition to the one already present, or the original set is resorbed and two new sets are differentiated; in either case, constriction then gives rise to two equivalent individuals.

It is now well established that each of the subdivisions of the ciliate body participates in morphogenesis. The essential role of the macronucleus in regeneration or fission has already been demonstrated by the early pioneers of experimental protozoology (Nussbaum, 1884; Gruber, 1886; Balbiani, 1889; Verworn, 1892; Johnson, 1893; *et al.*). Their observations have been confirmed for virtually every species subsequently studied. In contrast, the micronucleus has proved non-essential in a majority of cases. In some species, the presence of both micro- and macronucleus is apparently required for several or all phases of morphogenesis (data unreliable in certain instances; *cf.* review by Balamuth (1940).

As primary site of morphological expression, involvement of the ectoplasm in differentiation appears evident. Special functions of the cortical infraciliature have come to light through the pioneering work of Chatton, Fauré-Fremiet, Lwoff, and their collaborators (*cf.* reviews by Fauré-Fremiet, 1948; Lwoff, 1950), and through recent extension of their findings by Weisz (1951a, 1951b). Infraciliary kinetosomes are now known to act as local "organizers," uniquely determining the specific course of morphogenesis. This work has also resulted in the important realization that differentiative activity in ciliates, regardless of the verbal designation of the process as "regeneration," "excystment," etc., is analyzable in terms of a single fundamental pattern of events, in which kinetosomes play a decisive role.

The flowing endoplasm not only provides raw materials and energy sources for differentiation in progress, but through its initial metabolic condition also delimits the scope of differentiative activity. Thus the initiation or non-initiation of regeneration, for example, is determined in part by the amount and the types of reserve metabolites present (Weisz, 1948). Endoplasm, moreover, has an important, as yet only poorly understood function as a principal effector of form regulating movements and architectural rearrangements which characterize every morphogenetic event.

In the discussion to follow, evidence pertinent to the developmental function of both the nuclear system and the cytosome will first be reviewed. A conceptual synthesis of the conclusions will then be described.

THE NUCLEI IN DIFFERENTIATION

Both types of ciliate nuclei arise after fertilization from a synkaryon. During karyokinesis, micronuclei typically display chromosomes or chromosome aggregates, but macronuclei, dividing amitotically, do not. This generalization is probably justified, despite claims by some (*eg.*, Piekarski, 1941; Devidé and Geitler, 1947; Diller, 1948) that chromosomes can be seen in the macronucleus. Apart from the question of chromosomal organization, convincing evidence is available (*cf.* Sonneborn, 1947; also below) indicating the existence of macronuclear genes.

In its broad scope, the dichotomy of function of the two types of nuclei is well known. The micronucleus is primarily a germinal nucleus, largely non-essential except during karyokinesis. Somatic functions are principally under the control of the macronucleus. Four lines of evidence support this view.

1. Amicronucleate races are known for a large variety of species. Individuals of these races are normal, reproduce somatically for long periods, and remain capable of carrying out morphogenetic processes. Amicronucleate individuals are often less vigorous than micronucleate ones. The micronucleus presumably ensures optimal efficiency in the carrying out of somatic functions, but its presence is evidently not essential.

2. As already noted, the macronucleus alone suffices for regeneration in many species. In forms in which the micronucleus appears to be required in fission, but not in regeneration, the strong possibility exists that purely morphogenetic aspects of fission (that is, the differentiation of a double organism) are entirely under macronuclear control, and that the micronucleus is necessary primarily for divisional processes (that is, mitosis, constriction into two single individuals). In some species, however, both nuclei appear to be required for regeneration also. If the data are reliable, the possibility cannot be excluded that, in certain species at any rate, the micronucleus retains a somatic function.

3. The macronucleus, normally degenerating during or prior to fertilization, has never been found capable of acquiring a germinal role. In the mating of amicronucleate animals of Kahlia, Horvath (1947) finds small macronuclear fragments which give rise to new macronuclei. He regards these fragments as micronuclei. But since the organisms remain amicronucleate, and meiosis is not observed, his contention is unwarranted.

4. The most convincing evidence for the differences in nuclear function has been furnished by Sonneborn (1946). During the fertilization of *Paramecium aurelia*, the old macronucleus often fails to degenerate completely, but breaks up into a number of bodies, some of which later redevelop into normal macronuclei. Under such conditions, the synkaryon does not produce new macronuclei (Sonneborn, 1940, 1941). If such an animal, homozygous recessive for the killer gene *k*, is crossed with an animal homozygous dominant for this gene, the micronuclei arising in the former animal and in its progeny will be *K/k*; but the macronuclei, derived from the partial degeneration products of the old macronucleus, will be *k/k*. Under such

circumstances, the individual is found to remain non-killer. Evidently the phenotype is under macronuclear, not micronuclear, control. While micronuclear genes may remain active during vegetative stages, they are apparently unable to override the genic effects of the macronucleus.

This experiment reveals that each of the breakdown products of the old macronucleus contains at least one complete set of genes. In view of such evidence, Sonneborn (1947) concludes that the macronucleus normally contains many discrete subnuclei, each possessing a full genome. In this sense, the ciliate macronucleus may be considered "polyploid," as has been claimed by some, although "multinucleate" would be a more nearly correct term. It is not known whether individual subnuclei are actually polyploid or not. At any rate, most observers now subscribe to a polygenomal concept of macronuclear organization. In line with this view, amitotic macronuclear division implies the separation of two groups of subnuclei, achieved without a strictly quantitative karyokinetic mechanism. It may be noted that, historically, the probability of subnucleation has been implicit in numerous experiments on species in which the macronucleus can be fragmented by microsurgery: that single fragments retain the full potentiality of the whole nucleus has been discovered by the late 19th century protozoologists, and their work has later been amply substantiated.

Since the macronucleus arises from a diploid synkaryon, each subnucleus presumably is at least diploid. By comparing the relative amounts of nucleoprotein in the micro- and macronuclei of Paramecium, Moses (1950) estimates the latter to be approximately 40-"ploid." Such considerations point to a rapid development of numerous subnuclei, in the interval between anlage-formation and maturation of the macronucleus. Indirectly, evidence for this is provided by cytochemical observations on Blepharisma (Weisz, 1950a). These reveal a progressive increase in the concentration of polymerized DNA within the developing nuclear anlagen. The extent of DNA synthesis, delimited presumably by metabolic conditions, is also shown to be influenced by the competitive presence or absence of other macronuclear anlagen. Moreover, unless a given anlage can acquire a certain DNA content it cannot persist, but degenerates. These observations may indicate that a critical degree of subnucleation must be attained before a macronucleus can become fully functional.

Of the two nuclei, the macronucleus clearly plays the more fundamental role in processes of differentiation. The focal question to be asked is whether all subnuclei are or remain genetically equivalent, and whether they contribute equally to morphogenesis and morphostasis. Earlier evidence on the equivalence of macronuclear fragments (v.s.) appears to warrant an affirmative answer. However, recent experimental and cytochemical work on *Stentor coeruleus* and on *Blepharisma undulans* (Weisz, 1949b, 1950b, 1949c) leads to a different conclusion, yet does not refute the earlier data. In both species, micronuclei have proved non-essential for regeneration (Schwartz, 1935; Moore, 1924); both possess a single moniliform macronucleus composed of a longitudinal chain of nodes. Experiments on the two species are parallel, and those on *S. coeruleus* will be reviewed.

During the early stages after fission, all macronuclear nodes are equipotential, and each supports normal morphogenesis and morphostasis. Nodal capacities have been tested by excising all but a single desired node from individuals at given vegetative stages, and by observing whether this node can mediate regeneration, or can maintain the differentiated stage of the animal. Appropriate control experiments have been carried out simultaneously. By these means it has been found that as the vegetative cycle progresses, the differentiation potential of posterior nodes gradually declines. The decline is the more pronounced the more posteriorly a node is situated. Thus approximately midway through the vegetative cycle, the most posterior nodes support only partial regeneration: a gullet is not formed, and the peristome develops incompletely; or in originally intact organisms, the gullet is rapidly resorbed and the peristome degenerates partially. These conditions persist until death from starvation supervenes. Prior to fission, near the end of the somatic cycle, posterior nodes no longer mediate regeneration at all, and intact individuals dedifferentiate the entire oral apparatus. At this stage, mid-nodes still support partial regeneration, and as at any other stage, anterior nodes are fully active. During the ensuing fission, the macronuclear chain coalesces, and the fusion mass later reelongates, renodulates, and splits into two chains prior to cytosomal constriction. Nodes thus newly formed are again fully active, regardless of relative position. It has been found that, no matter how the fissional fusion nucleus is reoriented or fragmented, those nodes, and only those, which later come to occupy posterior positions, always lose morphogenetic activity. Moreover, once decline of activity has begun, experimental relocation of a node into an anterior position does not halt the decline, nor does it effect a return of the former potential. Such repotentiation is apparently achieved only if a node coalesces with others, and new nodes are modelled from the mass.

These data have led to the following conclusions consistent with the results as a whole. (1) Fissional condensation of the macronucleus probably entails a pooling of all morphogenetically active nuclear agents into a fusion mass. During renodulation, these agents would be distributed more or less evenly throughout the macronuclear substance. (2) The specific presumptive location of a nodal portion of macronucleus is determined largely by chance. (3) The cytosome at different antero-posterior levels apparently has a differential effect on the nodes, allowing retention of full activity at anterior loci, but effecting a progressive decline more posteriorly. (4) The decline of nuclear potential is irreversible, but coalescence of nodes brings about a reactivation of the critical agents. Thus nodes newly formed during fission are equipotential temporarily, regardless of position.

A precise parallelism could be demonstrated between the experimental data and the cyclical metabolism of highly polymerized DNA. Cytochemical methods have shown that the protein content does not vary appreciably from node to node or from stage to stage. The Feulgen reaction is equally constant in all nodes and at all stages. But application of Pollister and Leuchtenberger's (1949) critical methyl green technique reveals consistent variations in DNA concentration. Prior to fission, the macronucleus displays an antero-posterior gradient of concentrations paralleling precisely the morphogenetic gradient. Analogous correlations exist for all other vegetative stages. Supplementary experiments on hydrolytic breakdown and on extraction of nucleic acids, as well as Pollister and Leuchtenberger's interpretation of methyl green data, warrant the conclusion that decline of nodal potential is probably due, at least partly, to local depolymerization of thymonucleic acid; and that restoration of the potential during nuclear coalescence may be a result of repolymerization of the degradation products. It may be noted in this connection that Seshachar, (1950) emphasizing the chemical heterogeneity of the macronucleus in Chilodonella, is also led to infer the presence of different polymeric forms of DNA.

As pointed out above, parallel evidence, on both the experimental and the cytochemical level, has been adduced for B. *undulans,* and analogous conclusions are warranted. In this species, nodes destined to be inactivated are always situated near mid-body, in the posterior half of the organism. Unlike chromatin in *S. coeruleus,* which persists even when inactivated, decline of nodal potential in Blepharisma is accompanied by a gradual resorption of the affected nodes. Changes in morphogenetic activity can thus be gauged visually. Anterior and posterior nodes persist, and by augmented growth compensate for the loss of mid-nodal chromatin.

The "morphogenetic agents" with which these data are concerned may be equated to the macronuclear subnuclei: if the agents were argued to be subnuclear derivatives, the latter would have to be assigned precisely those properties that characterize subnuclei themselves (for example, spatial definition, initial completeness of genome, presence of numerous unit-genomes within the macronucleus). In substance, therefore, the data imply that in each somatic cycle, initial subnuclear equivalence gradually gives way to patterned genetic divergence.

In *S. coeruleus,* non-equivalence of macronuclear fragments is not likely to be detected unless specific attention is given to the exact site of fragment origin, and to the exact cycle stage at which a fragment is tested. Thus all earlier microsurgical examinations of macronuclear capacity, based on an essentially statistical premise, failed to reveal the changing potentials. Among numerous random experiments, a small number of cases would probably be found in which non-regeneration and death are due to nuclear insufficiency. But this causation would not be apparent, and such cases would merely be added to the general category of "experimental mortality." In B. *undulans,* nodal resorption has been described earlier (Young, 1939). The process has been thought to indicate macronuclear "purification." In a sense, this is a close circumscription of the present genetic implications.

In species in which the macronucleus remains permanently compact (for example, Paramecium), experiments analogous to those described above cannot be performed. Microsurgical methods can therefore not be employed to determine whether, at certain stages, genetic differences exist among the subnuclei, or whether subnuclear equivalence is maintained permanently. Conceivably, the latter possibility could be a result of the continued state of nuclear condensation: conditions such as are provided temporarily in the fission nucleus of *S. coeruleus* would exist constantly, and genomes would remain fully activated throughout the vegetative cycle. Either alternative is consistent with the conclusion regarding the equipotentiality of nuclear fragments in Paramecium, implicit in Sonneborn's experiments on macronuclear function. The fragments, when tested at the time of fertilization, are derived from a skeined reorganization nucleus which would be absorbed ordinarily. Even if local genetic differences existed, skein formation would obliterate them, as is the case in the nuclear reorganization of *S. coeruleus.*

For Stentor and Blepharisma, intranuclear genetic divergence is noted to occur in accordance with a consistent, predictable, spatial and temporal pattern. It has been concluded above that this pattern is imposed by properties of the cytosome. Thus before the implications of the nuclear changes can be examined further, the morphogenetic role of the cytosome must be evaluated.

THE CYTOPLASM IN DIFFERENTIATION

Among cytoplasmic organelles, the "infraciliature" of Chatton and Lwoff (1935) is of chief interest in the present context. The infraciliary complex is composed of subpellicular, ectoplasmic granules and fibrils, demonstrable visually by a number of silver techniques. Microanatomically, the basal granules of cilia form Chatton and Lwoff's "kinetosomes." A row of kinetosomes is paralleled on the right by an associated fibril, the "kinetodesma." The precise nature of the association, apart from the fact of proximity, is as yet undetermined. Fibril and granular row constitute a "kinety," and all kineties of the organism comprise the infraciliature.

The known functions of the infraciliary components may be summarized as follows.

1. Kinetosomes are self-reproducing particles, endowed with genetic continuity (Lwoff, 1950). They cannot arise *de novo.* Kineties increase in length by kinetosomal reproduction. Localized lateral reproduction of kinetosomes may occur, resulting in the formation of extrakinetal fields. If lateral reproduction takes place over the whole extent of the kinety, newly formed kinetosomes may organize into a new kinety, interpolated between two existing ones.

2. Kinetosomes are pluripotent morphogenetically. Directly or as intermediate agents , kinetosomes are required for the differentiation and maintenance of: trichocysts, flagellae, cilia, ciliary derivatives such as membranelles, cirri, undulating membranes (Lwoff, 1949, 1950); contractile vacuoles, gullet vestibule, holdfast organelles (Weisz, 1951a). Other

kinetosomal functions are likely to be revealed in time. Even at present, the list tends to bear out the conclusion that most, if not all, differentiations of the ectoplasmic cortex owe their origin and maintenance to the kinetosomes. (The granules constitute elements of the fibrillar system: this system might also be under kinetosomal control.)

3. Exercise of kinetosomal function is contingent upon the supply of endoplasmic metabolites (Lwoff, 1950). Raw materials and energy sources are required in differentiative activity, whether kinetosomes primarily direct the proper utilization of these substrates (synthesis of cilia), or whether they form building blocks in the emerging organelle (formation of trichocysts). In this connection, the ectoplasmic proximity of mitochondria (Fauré-Fremiet, 1910; Weisz, 1950c) and kinetosomes may possibly be significant.

4. Certain kineties are usually specialized in that they, and only they, normally participate in the differentiation of more complex organelles. Fauré-Fremiet (1948) discusses five general patterns among ciliates which exemplify this rule. In one of these patterns (displayed by *S. coeruleus*), all kinetosomes organized into kineties differentiate and maintain cilia, the most characteristic result of kinetosomal metabolism. But only those kinetosomes which are elements of kinety I, the stomatogenic kinety, can in addition initiate the differentiation of the more complex oral organelles (or the contractile vacuole, or the holdfast, Weisz, 1951a). Moreover, in *S. coeruleus*, kinety I alone has been shown to be the system of reference in relation to which the specific polarity of the entire organism is determined.

New kinetosomes arising by division within kinety I acquire the specialized kinetal potential. Unique morphogenetic capacities thus are retained permanently in the kinety.

5. In contributing to complex differentiations, kinetosomes within kinety I first reproduce. The original member of each pair of granules remains *in situ* and continues to maintain a cilium. Newly formed granules participate extrakinetally in other morphogenetic processes. In the differentiation of oral structures, for example, a dense "anarchic field" of kinetosomes is first laid down. The field then reorganizes into double rows of granules, representing the infraciliary framework of the presumptive peristome (Lwoff, 1949, 1950).

6. Kinetosomes and kineties are fundamentally equipotential (Weisz, 1951a). In *S. coeruleus*, any portion of kinety I retains the potentialities of the whole kinety. If kinety I is removed entirely, another kinety acquires the specialized functions. Moreover, within kinety I, every locus possesses a multiple potential. By choice of appropriate conditions, a locus can be induced predictably to differentiate either membranelles, or simple cilia, or a contractile vacuole, or a holdfast organelle.

7. These and related studies, as well as grafting experiments in this species (Weisz, 1951b), reveal the existence of an ordered hierarchy of "dominance" relationships among kinetosomes. In the presence of the kinetosomes of the oral organelles, for example, stomatogenic potentialities

of any other kinetosomes remain inhibited. But as soon as oral kinetosomes are removed, kinety I initiates stomatogenesis. Similarly, kinetosomes of kinety I exercise dominance over the kinetosomes of other kineties. In kinety I, terminal kinetosomes are dominant over those bracketed within the kinety. If two or more sets of oral organelles and kineties I are introduced into a unified graft system, kinetosomes of the largest oral region inhibit the stomatogenic potential of all other oral kinetosomes, and smaller oral structures dedifferentiate. Analogously, the largest kinety I dominates over other kineties, whether these had been specialized originally or not. These interrelationships clearly parallel the different degrees of structural and metabolic complexity of given organelles. From a purely quantitative standpoint, a larger organelle is evidently more complex than a similar smaller one. Also, in line with Lwoff's (1950) views, a greater organizational complexity may reasonably be postulated for kinetosomes maintaining membranelles, for example, than for those maintaining simple cilia. That this assumption is justified is shown in part by cytochemical tests on *S. coeruleus* and *B. undulans* (Weisz, 1949d, 1950b; also unpublished data). These reveal greater chemical complexity in the oral organelles than elsewhere, estimated either in terms of concentration of a single metabolite (tyrosine, phosphatase), or in terms of variety of metabolites (mucopolysaccharides specifically present in peristome).

Consistent with the foregoing considerations, the following general conclusions emerge with regard to the role of the infraciliature in morphogenesis and morphostasis. (1) In the normal organism, regional diversity of the explicit morphology is due to a divergence of the morphogenetic pathways followed by different kinetosomes. In view of initial kinetosomal equipotentiality, selection of a specific pathway must be influenced by the regional characteristics of the ectoplasmic microenvironment (also Lwoff, 1950). (2) This canalization process results in an acquisition, or an accentuation, of specific organizational characteristics within the kinetosomes, that is, the latter become differentiated chemically. (3) Continued maintenance of these characteristics in turn determines or perpetuates the regional specificity of the local environment. This provides further morphogenetic channeling stimuli for any newly formed, as yet undifferentiated kinetosomes produced in the vicinity. (4) Certain loci in the ectoplasm thus acquire greater organizational complexity than others. This is likely to be reflected in differential competition for endoplasmic substrates. Consequently, the observed hierarchy of kinetosomal dominance may be an expression of a parallel hierarchy of metabolic advantages. (5) The morphostatic condition, maintained in this manner, may be upset by renewed morphogenesis through a number of circumstances; for example, through structural or physiological injury, or through the cyclic acquisition of specific metabolic states incident to fission. In each case, actual or "physiological" removal of a group of kinetosomes vacates that level in the metabolic hierarchy which had been held by them. (6) Groups of kinetosomes next lower in the hierarchy may thus utilize the unclaimed share of endoplasmic materials. As a result, rapid reproduction is made possible, and new kinetosomes are produced which initiate a resynthesis of the lost parts. Resynthesis remains in "correct" channels under the limitations of the specific properties of the microenvironment, and also through the inhibiting barriers imposed by the remainder of the metabolic hierarchy. (7) In the course of resynthesis, the generating kinetosomes gradually acquire the characteristics, and the metabolic status, of those infraciliary elements which are being replaced. Thus the formation of additional undifferentiated kinetosomes is slowed and finally halted. A *status quo* is attained eventually.

In substance, ectoplasmic differentiation appears to involve a highly autonomous series of reciprocal interactions between kinetosomes and the local microenvironment. The "directed" character of a given differentiation sequence probably has its foundation in a morphostatic pattern which either pre-exists (as in fission), or is residual (as in regeneration); and in the self-limiting properties of the morphogenetic process, without which morphostasis would be impossible.

INTERACTION OF MACRONUCLEUS AND KINETOSOMES

Kinetosomal autonomy in morphogenesis, and somatic activities generally, are ultimately dependent on macronuclear functions. Evidence for this is ample, and some of it has been set forth in preceding sections. Similarly, a general reverse dependence of nuclear activities on cytoplasmic functions is revealed by various well-known phenomena. For example, cytoplasmic injury, starvation conditions, or the attainment of certain body volumes, soon lead to nuclear changes (for example, coalescence, division, or increased output of secretions). Recently it has also been shown that kinetosomes, particularly those of the developing adoral zone and the anarchic field, influence the kinetics of the macronucleus, bringing about either the initiation and continuance, or the inhibition, of nuclear coalescence (Weisz, 1951a). Lwoff (1950) has emphasized generally that cytoplasmic aspects of differentiation precede the associated nuclear changes. These cytoplasmic influences, however, have no direct bearing on the genic activity of the macronucleus.

Yet certain considerations, and the results of experiments on *S. coeruleus* and on B. *undulans* (Weisz, 1949b, 1951a, 1949c), appear to indicate that given genic activity of the macronucleus might in fact depend on the functioning of specific kinetosomes. The pertinent data are as follows.

In Stentor, experiments testing the morphogenetic potential of macronuclear nodes have demonstrated the progressive development, in the course of a somatic cycle, of a predictable pattern of subnuclear diversity. It has been concluded that this change is due to a differential antero-posterior effect of the cytosome. What property or entity of the cytosome could be responsible? Since varying nuclear potential is expressed principally in terms of oral morphology, and since oral organelles constitute the major distinguishing feature of the anterior region, it appears probable that the differential effect on the nucleus is due to the presence of oral components in one cytosomal region, and absence of such components from another. And as shown above, the essential elements of the oral organelles are the specialized oral kinetosomes.

In Blepharisma, similarly, one group of specialized kinetosomes is associated with the oral region, located in the anterior third of the organism (the peristome reaches almost to the anterior tip of the animal and the posterior peristomal limit is sometimes near mid-body, most often within the anterior third of the animal). Anterior macronuclear nodes, in juxtaposition to this oral region, persist throughout the vegetative cycle. In addition, a second group of specialized kinetosomes is probably situated near the blunted posterior end of the organism and is associated with the complex system of contractile vacuoles. As has been shown experimentally (Weisz, 1951a), the formation and maintenance of contractile vacuoles depends on the activity of specialized kinetosomes located in or near the free terminals of specialized kineties. In Stentor, vacuole and associated kinetosomes are located at anterior kinetal terminals, where they are in close proximity to the oral kinetosomes. In Blepharisma on the other hand, the system of contractile vacuoles, much more complexly differentiated than in Stentor (Moore, 1934), is situated posteriorly. It may be inferred that the generating kinetosomes derive from the posterior kinetal terminals. Thus posteriorly, much as anteriorly, persistence of macronuclear nodes appears to be correlated with the presence of specialized kinetosomes in the vicinity.

A series of experiments tends to substantiate the implications. If it were correct that oral kinetosomes affect anterior nodes so as to maintain full nuclear activity; and if decline of stomatogenic potential in posterior nodes of Stentor, or in mid-nodes of Blepharisma, were indeed due to the absence of specialized kinetosomes from posterior (or middle) regions: then anterior nodes transposed into posterior (or middle) regions should decline; and posterior (or mid-) nodes, transposed anteriorly before their normal decline has begun, should retain full activity. Experiments of this type, carried out on *B. undulans*, confirm the principle of the hypothesis fully. A strict correlation is evident between, on the one hand, maintenance of nuclear function and proximity of highly specialized kinetosomes, and on the other, decline of nuclear function and absence of specialized kinetosomes from the immediate vicinity.

These data suggest that highly specialized kinetosomes may specifically affect macronuclear nodes in the vicinity so as to allow full nuclear function to be retained; less highly differentiated kinetosomes do not appear to have this effect. What might be the nature of such kinetosomal influences? Similarly, how does the nucleus affect the kinetosomes? These reciprocal interactions may be circumscribed broadly by the following.

In the absence of direct contact, and in view of the interposition of endoplasm which is constantly flowing, the physiological connecting link between macronucleus and kinetosomes may be postulated to consist of diffusible substances, carried in either direction by endoplasmic currents.

It is clear that, in *S. coeruleus* for example, inactivated posterior nodes are inactive only from the standpoint of stomatogenesis: such nodes still suffice to differentiate and to maintain simple cilia (Weisz, 1949b; cilia cannot be differentiated anew in a wholly amacronucleate fragment, as repeated observation has shown). Macronuclear subnuclei must therefore contain agents required for cilium synthesis, distinct from other agents mediating stomatogenesis. Again, partially inactivated nodes cannot maintain a gullet and the proper geometrical alignment of the peristome; but the nodes suffice for the maintenance of peristomal components. Thus the existence of agents for "peristomal components" is indicated, distinct from agents for "gullet and peristomal organization." Reasoning along such lines leads to the conclusion that subnuclei may exist in at least as many different morphogenetically active states as there are possible levels of kinetosomal function.

Two major alternatives arise with regard to the possible nature of these different states. First, subnuclei unable to mediate stomatogenesis, for example, may lack the genes or the gene-products requisite for this process. Nodal inactivation might, in other words, be equivalent to differential and progressive inactivation of successive gene-groups. Second, the different morphogenetic states of the subnuclei might be an expression of a quantitative phenomenon. Conceivably, several distinct levels of overall nuclear activity might exist, not due to differential activity of given gene-groups only, but due to altered effective activity of all gene groups. As a result, the quantity, or the potency, of the subnuclear product might be reduced. The first alternative presupposes that the different demonstrable steps in stomatogenesis are under the control of different genes, the second alternative that they are mediated by the same genes, which would act at different effective levels.

Data concerning the different degrees of polymerization of DNA are probably consistent with either view. However, the following consideration perhaps tends to make the second alternative the more plausible one. Maximal nuclear activity can apparently be maintained by different types of highly specialized kinetosomes. A posterior node of Blepharisma, probably maintained in activity through the agency of kinetosomes associated with the vacuolar system, is morphogenetically fully equivalent to an anterior node, maintained by oral kinetosomes. Either node can mediate the differentiation of both oral and contractile organelles, as well as of any other ectoplasmic structure. Thus it would appear that once a kinetosome has attained a certain degree of differentiation, irrespective of the specific path of its differentiation, it may exercise a "maintaining" effect on all active entities of the macronuclear subnuclei. If progressive nodal decline would occur through inactivation of specific genic groups, then a posterior node of Blepharisma should soon lose its stomatogenic and every other but the vacuolar potential; and an anterior node should lose all but the stomatogenic potential. In actuality, however, both types of nodes retain their total potential. Thus, although present evidence is by no means conclusive, it nevertheless tends to indicate that a number of distinct levels of genomal activity may exist, each of which produces a distinct maximal morphogenetic effect. As noted above, these different levels of activity are characterized by different degrees of polymerization of DNA. The latter might be a reflection of different chemical states of genes themselves, or of degrees of ploidy, or of differences in the immediate genomal product, or of other related entities.

Nuclear effects would be mediated by the production of origin-specific substances carried in the endoplasm. Continued expression of a specific kinetosomal potency would then be dependent on the availability of a specific nuclear derivative. Variously differentiated kinetosomes in turn would release origin-specific substances in accordance with their degree of differentiation. The least specialized, that is, the ciliary, kinetosomes, mediate the retention of only the ciliary potential of the subnuclei. Membranellar kinetosomes effect the perpetuation of the nuclear potential to produce membranelles, and thus to produce cilia. Thus, if the genome is to persist at maximal effectiveness, at least a number of the most highly specialized kinetosomes must be present, or must be regenerated.

How could oral kinetosomes in Stentor maintain, for example, stomatogenic potential in anterior nodes, but not in posterior ones, considering that the "oral" kinetosomal factor would be distributed throughout the organism by the endoplasm? The experiments suggest that just after fission, when all nodes are still equipotential, anterior subnuclei, by virtue of their proximity to the oral region, might be the first to receive the kinetosomal agents, or might receive the largest share. Conceivably, this could establish an initial competitive advantage. Once gained, perpetuation of such an advantage would thereafter not depend strictly on proximity to the oral region. As posterior subnuclei are inactivated due to decreasing availability of the kinetosomal factor, antero-posterior competition would become increasingly unequal, and this would reinforce the initial disparity. In contrast, a similar disparity would not arise with regard to the potential for simple cilia, inasmuch as cilia are distributed over the entire ectoplasm. The correlated kinetosomal factor would thus be amply supplied to all parts of the nucleus; much as the nuclear factor for "cilia," produced in every subnucleus, would be available to kinetosomes in any part of the organism. In general, the hierarchic relationships among variously differentiated kinetosomes appear to be paralleled by similar relationships among the subnuclei. Initial kinetosomal, as well as subnuclear, equipotentiality is followed by correlative diversification of both, in a reciprocally reinforcing cycle of activities predicated by the existing morphological pattern.

The specific role of kinetosomal agents on the one hand, and of nuclear agents on the other, may be delineated broadly. It has been emphasized earlier that the autonomy of kinetosomes is potentially considerable, being rooted partly in intrinsic organization, partly in reciprocal, self-directive interaction with the local micro-environment. Yet explicit expression of this autonomy is contingent on effective availability of endoplasmic substrates for differentiative syntheses. This suggests that the basic function of specific substances emanating from the subnuclei may be enzymatic, permitting proper substrate utilization on the part of the kinetosomes. Analogously, gene reproduction and the manufacture of genic derivatives require endoplasmic substrates, again suggesting an enzymatic role for the agents supplied by the kinetosomes. "Inactivation" of a subnucleus thus may signify inability of the genome to reproduce or to manufacture characteristic metabolites, leading either to polymeric degradation (as indicated for *S. coeruleus*), or to degradation and resorption (as in *B. undulans*).

DIFFERENTIATION IN PROTOZOA AND METAZOA

The analyses of preceding sections may be combined into the following concepts:

1. Regional diversity of the ciliate ectoplasm appears to be a result of reciprocal, self-limiting, self-reinforcing interaction between kinetosomes and the cortical substratum.
2. In certain species, regional ectoplasmic diversity produces a correlated regional subnuclear diversification, through the agency of origin-specific kinetosomal metabolites. Differential availability of the latter entails inactivation or non-inactivation of given subnuclei.
3. Consequent differential manufacture of origin-specific subnuclear derivatives reinforces the morphological pattern existing in the ectoplasm.
4. Obliteration of the nuclear pattern, as in fission, still leaves the ectoplasmic pattern operative as a "template" according to which the original nuclear pattern is remodelled in a subsequent vegetative cycle.
5. Regeneration of a "correct" ectoplasmic morphology, following its partial destruction, is substantially equivalent to the reestablishment of an ordered hierarchy among variously differentiated kinetosomes. This process requires the continued presence of at least a residual portion of the original hierarchy. The metabolic barriers which it imposes provide the necessary morphogenetic canalization. With the further provision of specific nuclear derivatives, patterned redifferentiation may occur.

Derived principally from observations on Stentor and Blepharisma, such a concept is probably applicable to ciliates as a group. Differences of detail are likely to be encountered. Thus in some species, a possible accessory morphogenetic function of the micronuclei is yet to be explained. In others, a permanently compacted macronucleus probably does not display regional subnuclear diversity. But all ciliates possess kinetosomes organized into particular morphological patterns. The present concept implies in essence, that under certain "decentralized" macronuclear configurations local changes in the genome can be produced by activities of the kinetosomes. Subsequent activity of the genome, altered in accordance to the local morphological pattern, then reinforces that pattern. But existence of a specifically altered genome is not essential for the maintenance or the re-establishment of ectoplasmic morphology. As long as the necessary nuclear derivatives are supplied, directed morphogenesis and continued morphostasis are primarily a consequence of prior morphostasis.

Kinetosomes are noted to have central significance as points of intersection between the microenvironment/kinetosome cycle, and the kinetosome/genome cycle. Caspari (1950) has labelled these granules "visible plasmagenes." It is doubtful whether this designation is justified at present. All that can be safely stated is that kinetosomes constitute integral components of the cortex, elements which in large measure circumscribe the differentiative powers of the ectoplasm. Thus, in line with Weiss's (1950) hypothesis, they are perhaps better regarded as demonstrable groupings of particular molecular species which, together with other, so far non-demonstrable groupings, define given cortical characteristics. It is undeniably true that many aspects of kinetosomal function point up a number of cytogenelike properties. Yet these differ in at least one important respect from the properties of known plasmagenes. To date, none of the latter has been shown to affect essential morphogenetic or morphostatic processes: a Paramecium without kappa particles is no less a Paramecium than one containing such elements. Thus if "plasmagenes" are postulated to play a role in morphogenesis, plasmagene data actually available probably cannot be adduced as evidence. A "plasmagene" designation of kinetosomes in Paramecium or elsewhere, consequently has little merit, but adds considerably to verbal confusion. Indeed, the mechanism of differentiation here described alleviates the need to postulate *bona fide* plasmagenes for every organism. A cortical system endowed with greater or lesser autonomy, and a genome which is potentially plastic, suffice to account for differentiation.

Is it justifiable *a priori* to extrapolate results on ciliates to other protozoa, or even to multicellular organisms? Baker (1948) probably argues correctly that the polyploid (multisubnucleate) ciliate is not equivalent to the "cell" of other protozoa or metazoa. This argument implies, and probably no other argument can deny, that the ciliate organism is comparable to the whole metazoan organism. As Lwoff (1950) states so aptly: "A multicellular organism has differentiated cells; a ciliate has differentiated parts." The basic distinction between metazoa and single-celled protozoa on the one hand, and ciliates on the other, is clearly that in the former a morphogenetic "unit" happens to be co-extensive spatially and temporally with a cytological unit, a biochemical unit, a physiological unit, etc. In ciliates, the morphogenetic unit has no distinct cytological counterpart. But such a unit evidently exists; it contains some endoplasm, at least one macronuclear subnucleus, and a portion of the cortex.

Could the present concept be applied to differentiation in metazoa? The following more or less obvious considerations tentatively suggest an affirmative answer.

The major conclusions of experimental embryology point to the probability that such range of development as a cell possesses is implicit in potentialities of the cell cortex (Weiss, 1950), whether through the agency of visible kinetosome-like particles (for example, the basal granules of ciliated cells), or whether through invisible molecular ecologies. That the cortex always possesses a certain degree of autonomy is indicated by the fact that surface membranes are self-perpetuating organelles, endowed at least with somatic continuity. Cortical autonomy is paralleled by the manifestation of regional differences in cortical organization, apparent at the earliest stages of metazoan development and becoming increasingly evident thereafter.

After the first cleavage division, the total genome becomes progressively "decentralized." Each somatic cell nucleus formally corresponds to a subnucleus of the ciliate macronucleus; germ line nuclei, where these can be identified, are equivalent to the micronuclei of ciliates. There is no evidence that level-by-level "segregation of developmental potencies" (Weiss, 1939) might not be paralleled by a correlated "segregation of nuclear potencies," through a mechanism similar to that described above. Such a process might play a considerable role in metazoan differentiation. The concept is not inconsistent with genetic and cytological evidence.

Reduction of subnuclear activity to one of several possible levels has been noted to be followed, during a subsequent karyokinesis, by reactivation and an increased stainability of nucleoprotein. In metazoan cells, reduction of genic activity in given cells might equally be followed by reactivation, during mitosis or meiosis. In the latter, enhanced chromatin stainability is known to occur. In a post-mitotic cell, the genome would be fully active initially. As in the ciliate, however, such a cell would soon reacquire the original level of nuclear activity, through the limiting barriers imposed by the surrounding morphostatic pattern. But if the cell is cultured in isolation, unaffected by the original morphostatic barriers, reactivation of the genome would remain effective beyond karyokinesis. Modulated behavior would then be possible (to the extent that certain fundamental genes may no longer be active at all: cf. Mather's 1949, distinction between major genes, oligogenes, and polygenes). This might be the case for loose or migratory cells of certain connective tissues. Such cells, surrounded by relatively large amounts of tissue fluid and existing in environments which are rather non-rigid structurally, presumably are not too strongly under the influence of local morphostatic barriers. Presumptive gametes also mature in relative isolation, being expelled from the generating tissues. The reactivated genome would thus persist beyond meiosis, and this would contribute materially to the differentiation potential of the zygote.

SUMMARY

1. The known morphogenetic functions of the ectoplasm, the endoplasm, and the nuclear apparatus of ciliates are reviewed. The conclusions are integrated into a general concept of differentiation. The basic arguments are as follows.

2. *a.* The phenotype in ciliates is shown to be principally under macronuclear control. Evidence is presented to indicate that the macronucleus contains many discrete subnuclei, each possessing a complete genome.

b. Experimental and cytochemical data reveal that the subnuclei are equipotential initially. In certain species, however, differently located subnuclei undergo divergent fates during the course of a vegetative cycle. Genic function is retained at some loci, but reduced or lost at others. This occurs in accordance to a predictable regional pattern, concluded to be due to a differential effect of the cytosome. In the course of macronuclear reorganization during fission, equipotentiality of all subnuclei is again restored.

3. *a.* Explicit expression of the differentiation potential in ciliates is known to be under the influence of highly autonomous organelles, the ectoplasmic kinetosomes. It is shown that these granules are pluripotent morphogenetically and fundamentally equipotential. In the course of morphogenesis, given kinetosomes become differentiated chemically.

b. The local microenvironment is found to determine the specific pathway of kinetosomal development. Variously differentiated kinetosomes in turn impress various regional characteristics on the cortical ectoplasm.

c. Consideration of a variety of experimental data leads to the conclusion that a characteristic morphological pattern is maintained by metabolic barriers and limiting conditions which canalize different kinetosomes into an ordered hierarchy of functions. Directed morphogenesis following partial destruction of the pattern is then a result of the reestablishment of this kinetosomal hierarchy. A mechanism is outlined by which this may be achieved.

4. *a.* Evidence is reviewed which indicates that diversification of the subnuclear genomes is probably brought about by the regionally correlated diversification of differentiated kinetosomes. Conversely, the altered subnuclear pattern reinforces the particular ectoplasmic morphology.

b. It is concluded that these effects are probably mediated by specific (presumably enzymatic) agents manufactured both in kinetosomes and in the subnuclei. Through these agents, given kinetosomes control the level of subnuclear activity, and *vice versa,* in a reciprocal, self-reinforcing, self-limiting cycle.

5. The general implications of the data are discussed in the light of modern hypotheses bearing on problems of differentiation. These considerations suggest that the concept presented may have tentative applicability both to protozoa and to multicellular organisms.

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"PADDLE": A SEX-LINKED, RECESSIVE GENE OF TRIBOLIUM CASTANEUM HERBST[1]

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The objective of this paper is to record the presumed spontaneous occurrence of a new mutation for the flour beetle, *Tribolium castaneum.* This mutation is of some genetic interest in that it constitutes, so far as we know, the first reported instance of sex-linkage among the Coleoptera. It also has potential utility for population research—a utility enhanced both by the availability of several other genes now described for the genus and by the fact that a considerable body of knowledge exists relative to the population ecology of Tribolium.

Genes, and a variety of genetic phenomena, have been reported for the beetle families, Coccinellidae, Chrysomelidae, Curculionidae, and Tenebrionidae. In addition, Smith (1950) has recently summarized in some detail the cyto-taxonomy of 24 families of Coleoptera, and has shown (1950a) that *T. castaneum* has 9 pairs of autosomes, a small X chromosome, and a minute Y chromosome.

Tribolium, a tenebrionid, is represented in the genetic literature by discussions of two genes for *T. castaneum* and one for *T. confusum.* Park (1937) described the gene "pearl" (*T. castaneum)* as an autosomal recessive which, phenotypically, removes black pigment from the central ommatidia of the eyes. Miller (1944), working with the same species, dealt with two body color "strains." He showed that a mutant gene, when homozygous, changed the normal dark-red color to black. The heterozygote was intermediate between the two homozygotes, or dark reddish-brown. A rate-effect on metamorphosis was correlated with the presence of the mutant, namely: the larvae of the normal strain developed more rapidly when reared on a nutritious diet than did those of the black strain. Park, Ginsburg, and Horwitz (1945) described the gene "ebony" for *T. confusum.* The effect of this gene, when homozygous, is to alter body color from the red-brown characteristic of the species to a color that is essentially black to the eye. Genetic analysis proved that the gene was inherited as a simple, autosomal recessive. Ebony beetles also displayed a fecundity significantly reduced by about 10 per cent below that of normal forms.

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These three genes of Tribolium are useful as "population markers" in the sense that their respective, somatic expressions are readily diagnosed by inspection. They share in common the feature that they influence pigmentation. The new mutant, now to be discussed, also is quickly differentiable but differs in that it affects antennal morphology rather than eye or body color.

Before advancing proof of the hypothesis that this antennal gene is inherited as a sex-linked recessive, it seems advisable to contrast briefly the normal antenna with that of the variant. Reference to fig. 1 will be helpful in this connection.

The normal antennae of *T. castaneum* are distinguished by the facts that they are clavate; that the scape and pedicel are not markedly differentiated as to size or configuration; that eleven segments comprise the entire structure, and that the distinct club is formed by the distal three segments. The mutant antennae depart from these characteristics in several ways. First, and of prime diagnostic value, is the point that the club always shows some degree of fusion. That is to say, the sutures between segments do not complete their circumferential paths and fail, thereby, to effect movable articulations. There is some variation between individual mutants in this respect. The variation centers in the fact that the club can become progressively more reduced, even to a place where its first proximal segment is represented only by a minute vestige. The right-hand sketch of fig. 1, however, depicts the typical appearance and can be taken, so to speak, as the type-form of the mutant.

There exists also an interesting, but not understood, difference between males and females. Microscopic examination of 214 mutants selected at random from homozygous, recessive stock cultures showed that the females (112 in number) invariably possessed all the eleven segments characteristic of the genus. On the other hand, the males (102 in number) had lost two segments, thus giving them a total of nine. This is not a completely precise statement because in some males there was doubt as to whether a tiny segment-remnant did, or did not, exist immediately contiguous to the club. But, in any event, it is quite certain that males differ from females in having fewer segments, and that the modal number for males is nine while females always have eleven. Thus, it would seem that, in the embryogeny of the mutant, antennal imperfections increase from proximal to distal with the first segments of the flagellum not affected while the more outward segments, including the club of course, are subjected to greater teratology. This suspected process is apparently carried further in the male than in the female as suggested by the difference in total segment number. With a little experience it is quite feasible to separate mutants, whether males or females, from normal beetles by macroscopic inspection.

The mutant antennae appear paddle-like, and, in keeping with the custom of drosophilists who frequently seem to enjoy a homey nomenclature, we hereby designate this mutant of *Tribolium castaneum* as "paddle."

GENETIC EVIDENCE

Paddle first came to our attention in September, 1948, when, during the course of routine census examination of a mixed population of *T. castaneum* and *T. confusum*, a single male displaying this character was noted. Although the focus of our laboratory is ecological rather than genetic, we are reasonably alerted to the possibility of spontaneously occurring mutations. If an atypical beetle is seen, especially one whose morphological anomaly is bilateral, it is segregated to await genetic study. Preliminary assay soon showed that the paddle effect was inherited, and, after a stock culture had been established, a series of replicated, single-pair matings was initiated. All matings were made in glass vials containing eight grams of "standard medium" (95 per cent sifted whole-wheat flour: 5 per cent Brewers' yeast), and maintained in incubators at a temperature of 29°C., and a relative humidity of 65 to 75 per cent. Using the notation "+" to denote type and "*pd*" to denote the mutant, the following genotypes were paired: (matings I and II first; matings III and IV, depending on the progeny of I and II, later).

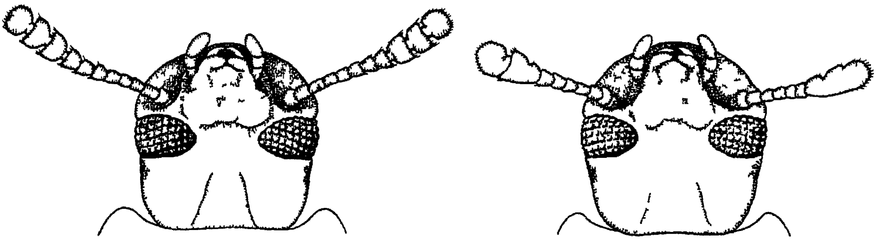

FIGURE 1. *Left*: Ventral view of the head of *Tribolium castaneum* showing normal antennae. *Right*: Same, but showing mutant-type antennae of male. (Drawings by Kenji Toda.)

I. Paddle male (*pd*/Y) × normal, homozygous female (+/+)
II. Normal male (+/Y) × paddle, homozygous female (*pd*/*pd*)
III. Normal male (+/Y) × normal, heterozygous female (+/*pd*)
IV. Paddle male (*pd*/Y) × normal, heterozygous female (+/*pd*)
V. Paddle male (*pd*/Y) × paddle female (*pd*/*pd*). (Stock cultures.)

The summed results from all replicates and all matings, and the ratios expected on the hypothesis that paddle is a single sex-linked recessive gene, are reported in table 1. Study of the table confirms the hypothesis and the following points may be arrayed in its support:

(1) Mating I yields only normal progeny in equal sex ratio. This follows expectation. The males are +/Y and the females are heterozygous +/*pd* as substantiated by the occurrence of paddle males among the offspring of Mating III. The excess of males (235) over females (202) is not a significant departure from 1 : 1 when tested by the point binomial (16.5 ± 10.4).

(2) Mating II yields 195 normal, heterozygous females (+/*pd*) and 185 paddle males (*pd*/Y). That the females are heterozygous is seen from the segregation of F_1

TABLE 1

OBSERVED PROGENY FROM FOUR MATINGS, AND PERCENTAGE PROGENY EXPECTED ON THE HYPOTHESIS THAT PADDLE IS SEX-LINKED AND RECESSIVE

Parental matings		Resulting progeny				Percentage of total progeny that are								Number of replicates
		Normal		Paddle		Normal				Paddle				
Designation	Genotypes	♂♂	♀♀	♂♂	♀♀	♂♂ (obs.)	♂♂ (expt.)	♀♀ (obs.)	♀♀ (expt.)	♂♂ (obs.)	♂♂ (expt.)	♀♀ (obs.)	♀♀ (expt.)	
I	*pd*/Y × +/+	235	202	0	0	53.8	50.0	46.2	50.0	0.0	0.0	0.0	0.0	8
II	+/Y × *pd*/*pd*	0	195	185	0	0.0	0.0	51.3	50.0	48.7	50.0	0.0	0.0	9
III	+/Y × +/*pd**	146	274	153	0	25.5	25.0	47.8	50.0	26.7	25.0	0.0	0.0	16
IV	*pd*/Y × +/*pd***	54	49	49	51	26.6	25.0	24.1	25.0	24.1	25.0	25.2	25.0	5

(* ♀♀ from Mating I. ** ♀♀ from Mating II.)

progeny in Mating IV into all four possible phenotypes. This finding agrees with the hypothesis. The sex and phenotypic ratio does not depart significantly from 1 : 1 (5 ± 9.7).

(3) Mating III yields the three expected segregants in the expected ratios of, respectively, ¼ : ¼ : ½, namely: 146 normal males (+/Y); 153 paddle males (*pd*/Y), and 274 normal appearing females, half of which are assumed to be homozygous (+/+) and half, heterozygous (+/*pd*). The observed numbers do not differ significantly from the calculated: for normal cf. paddle males, 3.5 ± 8.7; for all-males cf. females, 12.5 ± 12.0.

(4) Mating IV yields the four expected segregants in equal proportions: 54 normal males (+/Y); 49 paddle males (*pd*/Y); 49 heterozygous females (+/*pd*), and 51 paddle females (*pd*/*pd*). Again, the observed numbers do not depart significantly from the calculated expectation of 50.75.

(5) Mating V, or paddle male (*pd*/Y) by paddle female (*pd*/*pd*), has been maintained as a stock culture for over 25 continuous generations. It breeds true in that it produces, as expected, only paddle progeny.

It thus seems warranted to conclude on the basis of the above findings that the gene paddle is both sex-linked and recessive.

Finally, it is of some interest to examine, on a mean per vial basis, the total production of adult progeny within each of the four matings. These means are as follows:

Mating I: 54.6 ± 11.3 (C.V. = 58.6 per cent; d.f. = 7)
Mating II: 42.2 ± 8.1 (C.V. = 57.3 per cent; d.f. = 8)
Mating III: 35.8 ± 7.9 (C.V. = 88.3 per cent; d.f. = 15)
Mating IV: 40.6 ± 18.2 (C.V. = 86.0 per cent; d.f. = 4)

It is clear that no significant differences exist between any of the means. The greatest difference, that of (Mating I)-(Mating III), is but 1.4 times its standard error (18.8 ± 13.8). The production was also highly variable for all matings as attested by the coefficients of variability. These data cannot be taken as proving the non-existence of differential productivity since the experiments reported here were conducted, not to explore this question, but, rather, to obtain sex and character ratios. If desired, a more critical study could be readily designed to assay fecundity, fertility, and metamorphosis when crowding and intraspecies competition were minimized. But the mean values shown above do suggest that no striking trend in total adult production is correlated with parental genotype.

SUMMARY

A gene, designated "paddle," is described for the flour beetle *Tribolium castaneum*, and shown to be inherited as a single, sex-linked recessive.

The somatic effect of the gene is to cause varying degrees of fusion of the three segments constituting the antennal club. In addition, male mutant beetles show a reduction in the number of antennal segments from the eleven characteristic of the genus to a modal number of nine.

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A FURTHER ANALYSIS OF THE PANTOTHENICLESS MUTANTS OF NEUROSPORA

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During the last few years emphasis in the work on Neurospora biochemical mutants has been shifting from the use of these mutants as tools in determining pathways of biosynthesis to a study of the mutants themselves. Where previously it had been primarily a question of where the genetic blocks were located, it now seems more germane to inquire about the nature of these blocks. Since it has been assumed for many years, with good reason, that genes mediate chemical reactions through enzymes which they in turn control, the most feasible approach to the problem of genetic blocks is through a study of the enzyme systems in the mutant and wild type strains.

Initial attempts to distinguish enzyme differences between wild type and mutant, such as in the case of the adenineless mutant, 44206 by McElroy and Mitchell (1946), yielded inconclusive results. However, Mitchell and Lein (1948) investigated a tryptophanless mutant blocked at the reaction which couples serine and indole to produce tryptophane, and were able to show that cell-free preparations of wild type were active in this synthesis whereas those from the mutant were not. This result clearly indicated that the genetic block in question was due to the lack of an enzyme. It therefore provides support for the simple hypothesis that genetic blocks are due to the loss of enzymes *per se*. That is, loss of enzyme activity due to gene mutation is accompanied by the absence of the enzyme.

On the other hand, the work of Wagner and Guirard (1948) and Wagner (1949) on the pantothenicless mutants, 5531 and 34556 showed that the explanation of genetic blocks might not be quite so simple in all cases. It was found that in these mutants (which appear to be allelic or identical) the apparent genetic block is at the point of synthesis of pantothenic acid from β-alanine and pantoyl-lactone. The wild type is able to synthesize pantothenic acid from these precursors at a high rate *in vivo* in resting cell preparations but the mutant is not. When acetone powders are prepared from the mutant and wild type mycelia, these are found in both cases to be active in this synthesis. The mutant cannot carry out the synthesis *in vivo* under ordinary conditions of culture, therefore, but is able to do so under certain *in vitro* conditions. Obviously, the interpretation that genetic blocks are produced by simple loss of enzymes is not applicable in the case of the pantothenicless mutants, unless it is postulated that the acetone treatment of the mutant produced the enzyme artificially, as suggested by Horowitz (1949).

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[1] The Genetics Laboratory of the Department of Zoology.

It is the purpose of this present communication to report the results of additional work on the pantothenicless mutants, and to discuss their implications in the analysis of how genetic blocks are produced. It was assumed that if the mutant mycelium possessed the enzyme for pantothenic acid synthesis, it should be possible to demonstrate its activity under certain *in vivo* conditions. This assumption proved to be correct as is shown below.

EXPERIMENTAL

Synthesis of pantothenic acid in vivo:

The initial approach to the problem of inducing synthesis of pantothenic acid *in vivo* was to modify the culture conditions. It had been previously shown that a number of Neurospora mutants such as the riboflavinless and adenineless mutants could be caused to grow in the absence of riboflavin and adenine by changing the temperature (Mitchell and Houlahan, 1946a, b). The vitamin B_6-requiring mutant, 299, grows in the absence of B_6 at basic pH's (above 6.0) but not at lower pH values (Stokes, Foster and Woodward, 1943). Under acid conditions the B_6-requiring mutant, 44602, will grow on minimal medium provided the ammonium content of the medium is increased (Strauss, 1951). Attempts were made to stimulate growth of the pantothenicless mutants, 5531 and 34556, in the absence of pantothenic acid by varying the pH, temperature and nitrogen content of the medium. No stimulation was observed even when suboptimal amounts of pantothenic acid were added. Therefore, it was assumed that no pantothenic acid was synthesized. None of the conditions was so drastic as to prevent the growth of the mutants in the presence of pantothenic acid.

Following these negative results an attempt was made to determine if pantothenic acid is synthesized by the mutant after growth has been initiated by the addition of pantothenic acid. There are a number of cases reported in which biochemical mutants have responded by synthesizing the required nutrilite after growth had been started in the presence of the nutrilite. The riboflavinless mutant of Neurospora has been shown to be one of these (Mitchell and Houlahan, 1946a).

The mutants, 34556 and 5531, and wild type, 5256, were tested in flasks containing 50 ml of standard Neurospora minimal medium, some containing in addition the pantothenic acid precursors, 0.004M β-alanine and pantoyllactone. The mutant culture media were made complete by the addition of 18γ of pantothenic acid per flask. The flasks were incubated at 25°C and 37°C and pantothenic acid assays were made each day on a set of flasks using *Lactobacillus arabinosus*. The results of these assays, which were essentially the same for both mutants at both temperatures, are given in fig. 1 for 34556 and for wild type over an eight-day period at 25°C. The dotted line in this graph represents the average dry weight increase over

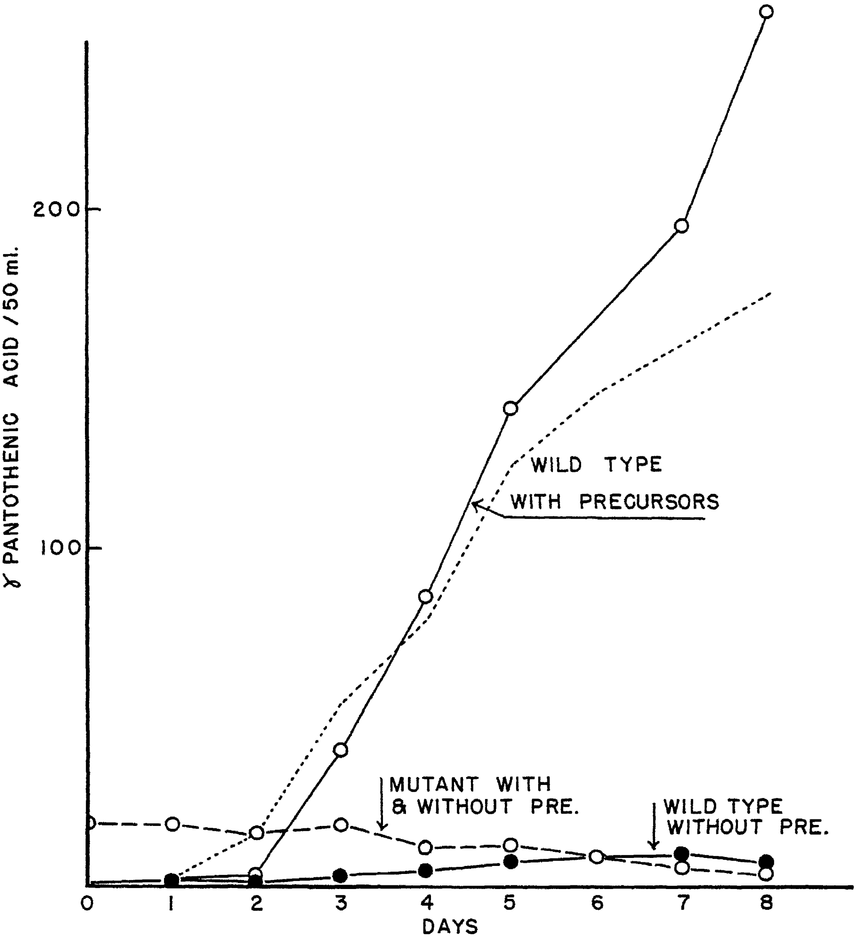

FIGURE 1. Production of pantothenic acid by wild type and mutants in standing culture.

the period observed. Actually assays were made each day for 13 days, but no change was noted in the amount of pantothenic acid present in the mutant culture media, whereas the wild type growing in the presence of the precursors continued to synthesize pantothenate, producing up to as much as 6 mg in 50 ml of medium. Wild type in the absence of the precursors produced no more pantothenate after 13 days than indicated in fig. 1 for an 8-day period.

To test the possibility that substances excreted into the medium by the pantothenicless strains inhibited the synthesis of pantothenic acid, mycelial pads were removed from some of the flasks on certain days, washed and incubated in the presence of 0.004 M concentrations of both precursors and phosphate buffer at ph 6.5 for 24 hours. The results are given in table 1. Included in this table are the values for pantothenic acid synthesis by the wild type mycelium. It is evident that the intact mutant mycelium has no enzyme activity. The intact wild type mycelium decreases in activity for pantothenic acid synthesis on about the third day. In addition, comparable amounts of pantothenic acid are synthesized by wild type whether or not the mycelium is grown in the presence of the precursors.

TABLE 1

PANTOTHENIC ACID SYNTHESIZED BY UNTREATED RESTING MYCELIUM OF VARIOUS AGES AFTER GROWTH IN STANDING CULTURE. γ PANTOTHENIC ACID/MG DRY WEIGHT MYCELIUM PER 24 HOURS.

	Age of mycelium in days				
	3	6	7	8	13
Wild type grown in absence of precursors	8.7	4.1	2.4	3.0	1.8
Wild type grown in presence of precursors	8.9	5.1	3.0	4.9	1.5
34556 grown in absence of precursors	0.02	0.02	0.01	0.01	0.01
34556 grown in presence of precursors	0.02	0.01	0.02	0.03	0.01

Attention was next directed to the possibility of synthesis under conditions of vigorous aeration. It had been found that a culture of mutant mycelium grown in a five liter volume of medium under constant aeration for five days had accumulated 51 mg of pantothenic acid. This meant that 41 mg of the vitamin had been synthesized in five days, since 10 mg were used as a supplement to start growth. Subsequent experiments under controlled temperature conditions verified this synthesis.

The mutants and wild type were cultured in 500 ml of medium in one liter round bottom flasks immersed in a water bath set at 30°C. A constant stream of sterile air was bubbled through the medium in each flask throughout the course of the experiments by means of a glass tube which opened near the bottom of the flask. The culture medium for the mutant was supplemented with 1γ of pantothenic acid per ml. Pantothenic acid assays were made on a 10 ml aliquot of each culture every day for eight days. Fig. 2 presents the data obtained for 5531, giving the amount of pantothenic acid present per ml of medium. Flasks were also incubated at 25°C with essentially the same results. The volume of medium in the flasks did not vary more than 10 to 20 ml from the 500 ml original volume during the course of the experiment. It was kept within this range by saturating the entering air with water and replacing the assay aliquots with an equal amount of sterile distilled water after each withdrawal. On the eighth day the mycelium in each flask was removed, squeezed dry, weighed, and a small

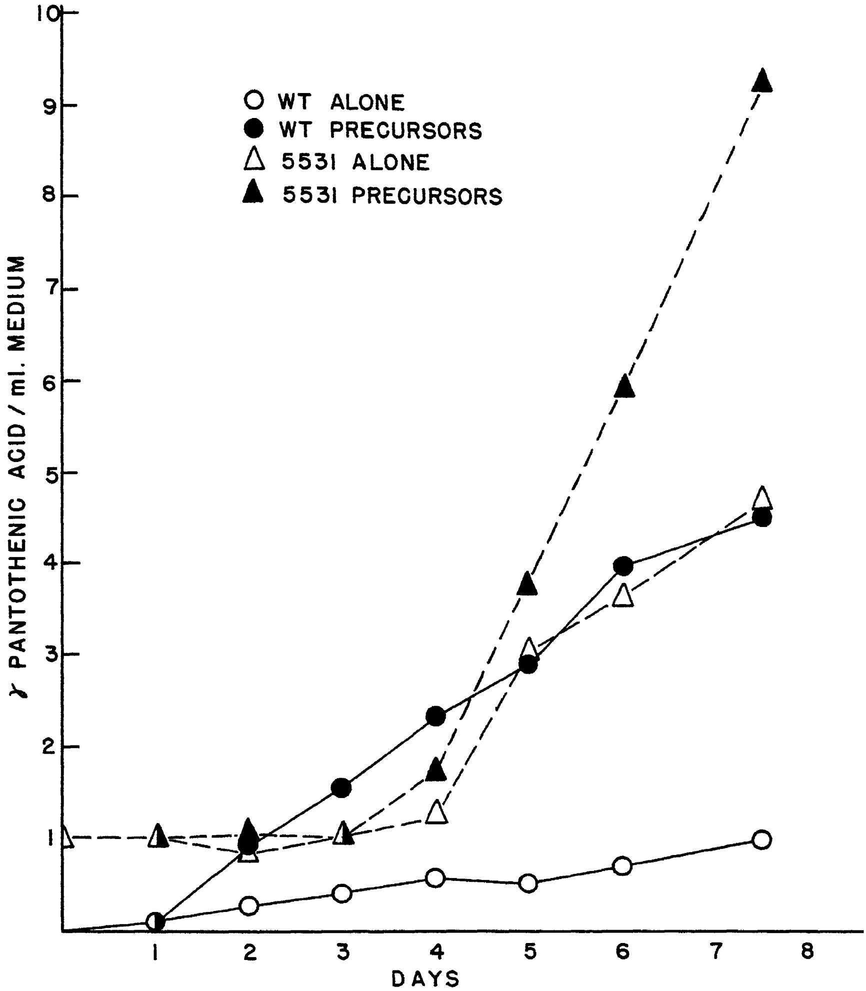

FIGURE 2. Synthesis of pantothenic acid by wild type and 5531 in an aerated medium.

sample dried and weighed. The remainder was blended with 0.1M potassium phosphate buffer at pH 6.5 and allowed to autolyze for 72 hours under toluene. The residue was discarded and the autolysate assayed for pantothenic acid. From this the pantothenic acid content of the mycelium was determined. The total pantothenic acid determined in the culture filtrate was added to this, after subtracting the pantothenic acid added originally to the mutant cultures. The total dry weight of the mycelium was determined and the amount of pantothenic acid synthesized per mg dry weight of mycelium was calculated. These results are given in table 2 for two independent runs using the above procedure. These values may be some-

TABLE 2

SYNTHESIS OF PANTOTHENIC ACID UNDER CONDITIONS OF AERATION. PANTOTHENIC ACID GIVEN AS γ PER DRY WEIGHT OF MYCELIUM AFTER 8 DAYS GROWTH.

5531 (mutant)			5256 (wild type)		
A Minimal only	B with precursors	B/A	A Minimal only	B with precursors	B/A
0.985	3.12	3.14	0.649	2.95	4.55
0.717	2.63	2.92	0.498	2.30	4.60

what low since pantothenic acid in bound form, particularly in the form of coenzyme A, is not assayable with *Lactobacillus arabinosus,* unless freed by the proper hydrolytic procedure. It is not known whether such hydrolysis occurred during autolysis of the mycelium.

Small amounts of resting mycelium grown under aeration were tested for activity in the presence of precursors and buffer by the procedure described previously (Wagner and Guirard, 1948). The results are given in table 3 for wild type, 5531 and 34556 cultured in the presence and absence of precursors. The activity of the mutant mycelium is evidently similar to that of the wild type. The activity of the wild type mycelium in this experiment is consistently higher than for the 8 day old wild type mycelium grown in standing culture. (See table 1.)

With regard to the observed activity for synthesis of pantothenic acid by the mutant cultures, the question arises as to whether there had been a back mutation to the normal at the pantothenicless locus, or a suppressor mutation. This possibility was tested by transferring small bits of mycelium from the mutant cultures into fresh minimal medium. No growth was observed in the inoculated flasks. On the other hand, growth was obtained if pantothenic acid was added as a supplement to the medium. It seems firmly established therefore that back mutation is not the explanation for the activity of the mutants. In this connection it is interesting to note that the pantothenicless gene seems to be a particularly stable one. Giles and Lederberg (1948) have performed extensive experiments to determine the back mutation rate of a number of biochemical mutants, and were unable to detect any for pantothenicless, although they found that other loci such as inositolless back mutated readily. The present authors have done similar experiments with pantothenicless and obtained similar negative results.

In the experiments previously reported (Wagner, 1949) in which pantothenic acid synthesis by the mutants was described, the evidence for the identity of the pantothenic acid synthesized by the mutant with synthetic pantothenate had been based entirely on the growth response of the mutants themselves and *Lactobacillus arabinosus.* While this is very good presumptive evidence that the two are identical it is not proof. Additional information as to their relationship was obtained by means of paper chromatography using a butyl alcohol-acetic acid-water mixture. The mutant-synthesized pantothenic acid has an R_f of 0.76 compared to 0.75 for synthetic pantothenate (converted to the free acid with oxalate before chromatography). The close similarity between the two R_f values makes it almost certain that the mutant produces pantothenic acid and not some other compound with similar activity.

COMBINATION MUTANTS INVOLVING PANTOTHENICLESS

Attention was next directed to the possibility of modifying the *internal* conditions of the mutant in order to suppress the phenotypic expression of the pantothenicless gene. The assumption was made that perhaps the enzyme necessary for uniting pantoyl lactone and β-alanine is inhibited by a compound or compounds produced by some other metabolic process.

In order to test the possibility that some naturally occurring compound synthesized by Neurospora might be responsible, a number of amino acids, vitamins, purines and pyrimidines were tested for inhibitory activity on the synthesis of pantothenic acid *in vitro*. None proved to be consistently inhibitory with the exception of cystine and betaine. Betaine was investigated further to determine its effect on the synthesis of pantothenic acid *in vivo* by wild type. In addition, tryptophane which definitely inhibits the growth of both wild type and pantothenicless was tested in the same way. Betaine proved to have no effect on wild type in concentrations up to 5 mg per 25 ml, but tryptophane showed a decided inhibition. In the absence of tryptophane, actively growing wild type mycelium produced 0.88 ± 0.06 γ of pantothenic acid per mg dry weight of mycelium from precursors in eight days, whereas in its presence (5 mg per 25 ml) only 0.54 ± 0.06 γ was synthesized per mg dry weight.

TABLE 3

SYNTHESIS OF PANTOTHENIC ACID BY 8 DAY OLD, RESTING, AERATED MYCELIUM

Strain	γ pantothenate per mg. dry weight mycelium per 24 hrs.
Wild type	6.8
5531	7.5
34556	7.7

The pantothenicless mutant 5531 was crossed to UT 1, a mutant which when originally discovered in this laboratory grew slightly on minimal but required tryptophane for growth equivalent to wild type. Ascospores from this cross were picked at random and tested for growth on minimal, minimal supplemented with pantothenic acid or tryptophane and minimal with both. Ascospore isolations from single asci were not feasible since there was too large a number of inviable spores. Wild type, pantothenicless and presumed double mutant segregants were identified. However, no spores of the parent UT 1 strain were recovered. It was assumed that these were possibly represented among the inviable spores. Cultures from viable spores which produced mycelium on minimal medium, but showed a stimulatory effect of pantothenic acid or tryptophane were assumed to be double mutants of UT 1 and 5531 and were tested by backcrossing to wild type. In a number of these both wild type and 5531 segregated in the same ascus sac but again UT 1 was not recovered. The results clearly showed, however, that 5531 was included in the double mutant strains. One of the double mutants, here designated as B3, was tested most extensively with respect to its growth requirements and ability to synthesize pantothenic acid. It is not only capable of synthesizing pantothenic acid, but its capacity for synthesis in the presence of the precursors and under conditions of aeration is from five to six times greater than the wild type and 5531.

UT 1, unfortunately, was practically sterile in crosses to wild type; only about 10 per cent of the spores resulting were viable, and all these proved to be wild type. It may be that UT 1 is not a simple point mutation, but a chromosome aberration. In addition to the aberrant behavior in crosses, UT 1 reverted to the wild type condition in so far as tryptophane no longer had any stimulatory influence on its growth. Because of this, and its sterility in crosses, experiments on this particular combination were discontinued.

The pantothenicless mutant was also crossed to UT39, an ultra-violet induced mutant which has been characterized in detail by Haddox (1951). UT39 grows only slightly (trace to 3 mg in 25 ml) on minimal medium during the first 72 hours, but after 72 hours its rate approaches that of wild type. In the presence of both phenylalanine and tyrosine together in equimolar concentrations of about 2×10^{-4} M, growth during the first 72 hours is almost identical to wild type. Tyrosine or phenylalanine stimulate growth alone, but not to as great an extent as in combination. Repeated backcrosses of this mutant to wild type have always resulted in a type of segregation characteristic of a single gene difference.

The double mutant obtained from the cross 5531 × UT39 was tested by backcrossing to wild type, and its constitution proven by finding that the mutant strains 5531, UT39 and the wild type and combination segregated in the expected ratios. The three types of ascus arrangements which occurred out of 16 dissected are as follows:

Ascus type	Spores							
	1	2	3	4	5	6	7	8
1	C	C	C	C	wt	wt	wt	wt
2	5531	5531	5531	5531	39	39	39	39
3	5531	5531	39	39	5531	5531	39	39

This double mutant has the following growth characteristics: on minimal medium it grows like the parent strain UT39; it is not stimulated by pantothenic acid, tryosine, or phenylalanine alone, but equimolar amounts of the latter two combined show definite stimulation (see tables 4, 5). Panto-

TABLE 4

GROWTH OF THE DOUBLE MUTANT (UT39, 5531) AND UT39 ON MINIMAL AND VARIOUS SUPPLEMENTS. MG DRY WEIGHT MYCELIUM AFTER 72 HOURS

Supplement	UT39	5531, UT39
none	5.0	1.5
tyrosine		
2×10^{-6}M	17	1.9
2×10^{-5}M	31	1.5
2×10^{-4}M	34	1.6
2×10^{-3}M	16	1.5
phenylalanine		
2×10^{-6}M	22	2.2
2×10^{-5}M	39	1.5
2×10^{-4}M	49	1.5
2×10^{-3}M	39	2.2
tyrosine and phenylalanine*		
2×10^{-6}M	19	1.5
2×10^{-5}M	30	6.5
2×10^{-4}M	40	2.7
2×10^{-3}M	21	3.6
pantothenic acid	4.1	1.6

*Range between 4×10^{-5} to 4×10^{-4} gives 20 mg or more growth of double mutant in 72 hours.

thenic acid and tyrosine combined have no effect on either UT39 or the double mutant, other than the expected tyrosine stimulation of UT39. The double mutant, then, shows a complete suppression of the pantothenicless phenotype, and in part the tyrosineless and phenylalanineless, since it does not respond to either of the stimulatory amino acids alone.

DISCUSSION

The original interpretation of the pantothenicless mutants of *Neurospora* as being due to the absence of the enzyme catalyzing the synthesis of pantothenic acid from β-alanine and pantoyl lactone (Beadle, 1945) is no longer tenable. It is now established that such an enzyme is present *in vivo* as

TABLE 5

GROWTH OF THE DOUBLE MUTANT (5531, UT39) AND UT39 ON MINIMAL AND VARIOUS SUPPLEMENTS. MG DRY WEIGHT MYCELIUM AFTER 84 AND 120 HOURS

Supplement	UT39		(5531, UT39)	
	84 hrs.	120 hrs.	84 hrs.	120 hrs.
none	10	40	3.2	40
20 γ/25 ml. pantothenic acid	8.2	40	6.2	39
2×10^{-4}M tyrosine	45	69	8.0	53

well as *in vitro* preparations, but is inactive under certain *in vivo* conditions. Alternative explanations for the genetic block in these mutants are therefore in order.

Since there is no other rational basis for hypotheses dealing with the primary or nearest observable action of genes evident at the present time, one must proceed on the assumption that genes mediate chemical reactions through enzymes. Starting then with the observation that the enzyme *per se* is present in this case one may interpret the results reported in this and previous papers by the following alternatives:

I. The mutation involves a gene directly concerned with the enzyme coupling β-alanine and pantoyl-lactone.

II. The mutation involves a gene controlling a reaction having nothing to do directly with the synthesis of pantothenic acid. This reaction results in the production of a compound which inhibits the enzyme catalyzing the synthesis of pantothenic acid.

Applying these hypotheses to the observed facts results in the following conclusions:

Considering hypothesis I first, we may conclude that any change brought about in the enzyme is not a simple quantitative change, since the synthesis of pantothenic acid by wild type and the mutant under proper conditions proceed at approximately equal rates. Nor, is there a simple qualitative change involving only an enzyme·changed in its affinity for its substrate. The extremely low activity of the mutant *in vitro* preparations at 25°C compared to the wild type, and to mutant activity at 30°C (Wagner, 1949) indicates a qualitative change, but there is no effect of increasing the temperature on the growth of the mutant in the absence of pantothenic acid. Furthermore, the mutant enzyme is active *in vivo* under aeration at 25°C. The change must involve the relationship between the enzyme and substances in the cell other than the pantothenic acid precursors. The enzyme in the mutant must be so altered that even though it is capable of carrying out the synthesis of pantothenic acid, it is prevented from doing so by the inhibitory activity of other substances. The affinity of the altered enzyme for the pantothenic acid precursors may be affected under certain conditions, but the more important result is that it is made subject to complete inhibition by other metabolites in the cell. These may be natural compounds such as proteins, amino acids, etc., which are involved in other phases of metabolism.

Using the first hypothesis, then, the activity of the *in vitro* preparations could be explained on the basis that an inhibitor or inhibitors are destroyed or removed in the process of preparation with acetone. The former would be more likely since concentrates prepared from the acetone washings do not seem to have any inhibitory activity on the synthesis *in vitro*. The demonstrated activity *in vivo* is more difficult to interpret, but one may assume that after an initial period of growth in aerated medium made possible by added synthetic pantothenate, the excess of oxygen or perhaps changes in CO_2 content bring about changes in certain phases of metabolism which result in part in a decrease of the inhibitor. Dagley, Dawes and Morrison (1950) have reported that *E. coli* and *Aerobacter aerogenes*, when grown in unaerated medium, excrete amino acids (tentatively identified as histidine, aspartic acid, glutamic acid and alanine) into the medium which are easily detectable by paper chromatography without recourse to concentration of the filtrate. Cells cultured in aerated medium, however, only produce these amino acids in traces. The filtrate must be concentrated ninety-fold in order to get visible spots on paper. Accompanying this difference in amino acid excretion it has been found that the concentration of pyruvic acid increases during the active phase of growth of an aerated culture, but falls rapidly to zero after the stationary phase begins. In unaerated cultures the concentration of pyruvic acid attained is higher and the fall in concentration is not pronounced after the stationary phase sets in. Comparable but not necessarily identical changes in metabolism might be expected in the case of Neurospora as a result of aeration. Preliminary experiments conducted to test this have indicated that such is the case, but more work along this line is in order before any definite conclusion can be drawn.

The double mutant (5531, UT39), which grows in the absence of pantothenic acid, would by orthodox genetic terminology be described as a case of suppression of the 5531 gene by UT39. This is a good description of a condition, but not an analysis. A complete analysis of the suppression would require a more or less complete understanding of the inherited metabolic upsets in each one of these mutants, and we do not have this understanding at the present time. However, if one accepts the hypothesis that 5531 is incapable of producing pantothenic acid due to an inhibition, and that this inhibition is relieved in the presence of the UT39 gene by the reduction in concentration of the active inhibitor, a working hypothesis may be set up which may be of value for future investigations.

The second hypothesis given above is related to the first in so far as an inhibition is postulated. The essential difference is that the pantothenate enzyme is not altered, but that a natural inhibitor produced in excessive amounts due to mutation at another locus completely inhibits the enzyme at the higher concentrations. Both hypotheses, therefore, center around the relationship between an enzyme and its inhibitor. Altering the enzyme qualitatively or the inhibitor quantitatively should produce the observed effects.

It is not possible with the present data to differentiate between the two alternatives, or even to state that they are the only two possible explanations. Other hypotheses may be formulated which will explain the above described facts, but in the opinion of the authors the two given are the simplest, and most susceptible to further experimental investigation.

It must be noted in conclusion that the results obtained here with double mutants are not the only ones reported which are in conflict with the enzyme absence hypothesis as a general explanation of the inability of an organism to produce a particular chemical compound. Houlahan and Mitchell (1948), Emerson (1948) and Mitchell and Mitchell (1950) have all described Neurospora double mutants of various types in which suppressors are involved. It is our opinion that proper combinations of mutant genes made by selected crosses between previously characterized mutants will result in a clarification of the concept that many biochemical mutants are due to internal upsets in the *balance* of metabolic systems. The object should now be to investigate the validity of this concept rigorously by combining unbalanced systems which complement one another and thus reestablish new balances. This is not heterocaryosis, but the synthesis of new balanced systems at a somewhat different level from the old. The evolutionary and biochemical implications of this concept are obvious.

SUMMARY

The pantothenicless mutants, 5531 and 34556, are active in the synthesis of pantothenic acid *in vivo* when the cultures are aerated. When 5531 is combined with the mutants UT 1 and UT39, the double mutants do not require pantothenic acid for growth. The bearing of these results on the analysis of genetic blocks is discussed.

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THE EFFECT OF NUTRITION ON THE PRODUCTION AND STABILITY OF AN UNSTABLE WHITE COLOR-VARIANT OF THE HY STRAIN OF SERRATIA MARCESCENS

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Recent studies with the Hy strain of *Serratia marcescens* have revealed a type of color variation which is not affected by mutagenic agents but which appears to be influenced by nutritional factors in the medium.

When suspensions of cells from typical Hy cultures were plated on peptone agar, color-variants were rarely seen and it was believed that the strain was relatively stable, but plating of the same suspensions on a synthetic ammonium citrate glycerol agar invariably gave a considerable number of white colonies which were conspicuously unstable and developed a few red spots after two or three days incubation. Because of the appearance of the colonies the variant was known as "speckled."

Variants of the speckled type were produced with great regularity by the Hy strain; 3-day old red colonies always contained about 1 per cent of cells of this type. Other spontaneous color variants were much less common and were relatively stable.

The speckled variants reverted rapidly to the pigmented type. When 3-day old speckled colonies were plated on synthetic agar they gave approximately ⅔ speckled daughter colonies and ⅓ red. Stable whites and intermediate pinks were not found.

Treatment of suspensions of red cells with ultra-violet light gave a great increase of other color variants (up to 30 per cent of the survivors gave colonies with heritable differences in pigmentation) but there was no change whatsoever in the proportion of cells that gave typical speckled colonies. Similar results were obtained by treatment with nitrogen mustard. The failure of mutagenic agents to affect the rate of production of the speckled variants, together with their high spontaneous frequency and their instability, clearly differentiated this type of variation from the others observed with the Hy strain.[1]

The number of speckled-type cells in synthetic cultures rose abruptly during the late lag and early logarithmic phases as found by plating aliquots on synthetic agar and observing the number of red and speckled colonies. This increase occurred even though the rate of multiplication of the speckled type in synthetic broth was lower than that of the red type. By means of marked strains it was clearly shown that the increased number of speckled-type cells did not come from the original white cells but from the red cells of the inoculum. During rapid growth in synthetic medium many of the progeny of the red cells had lost the ability to produce pigment.

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[1] The common spontaneous, reversible, dark-red to bright pink variation previously described (Bunting, 1946) in the 274 strain has also been found to be unaffected by mutagenic agents.

No such increase in white variants was observed in media supplemented with peptone or yeast extract. Moreover, analyses of cultures by plating showed that populations of white cells inoculated into such media were rapidly changed into the red type. The evidence from the rate of change of the color types and from the behavior of mixed populations of marked strains indicated that selection was not responsible for the observed shifts but that in the presence of the supplement the white cells regained their ability to produce pigment and were changed into the red type. Even 24-hour colonies from speckled-type cells when grown on agar supplemented with yeast extract were bright red and contained a majority of cells of the red type, although sister colonies on synthetic agar were white and often failed to reveal the presence of any red cells at this early stage.

Until further data are available there seems no advantage in choosing between the various hypothetical mechanisms which can be conceived to account for the variations described above. The production of white cells during rapid growth in synthetic media suggests the loss of a cytoplasmic factor similar to kappa in paramecium (Sonneborn, 1946) or perhaps cytochrome-oxidase granules in yeast (Ephrussi, 1949); but the Serratia variants differ significantly in their ability to revert to the pigmented type when conditions are favorable. Actually the type of reversible change observed in the Serratia cultures has much in common with the mass phenomena described by Spiegelman (1950) for long-term adaptation to galactose fermentation in yeast. Further studies are in progress to identify the nutritional factors essential to the shifts in color types which have been observed and to elucidate the mechanisms by means of which they are accomplished.

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LETTERS TO THE EDITORS

Correspondents alone are responsible for statements and opinions expressed. Letters are dated when received in the editorial office.

ON MOSAIC INHERITANCE IN MAMMALS

Dr. W. E. Castle, in his article, "Are Mosaic Genes Semi-allelic?"[1], has commented on my article 2. He refers to the mosaic inheritance known in mammals as conforming only partially with the concept of semi-allelic genes presented in my article. In the "Japanese" rabbit, for instance, the black and red areas make a mosaic, and the whole behaves as a unit character which is alternative to either black or red. He interprets this to be due to "a reorganization of the gene molecule (or whatever it is that constitutes a gene) so as to include both alternative determinants side by side yet inseparable." It seems to me that the Japanese pattern is produced by the gene for black color (*E*) and the gene for red color (*e*) arranged in direct sequence on the same chromosome. These genes are so closely linked as to allow practically no crossing over. In the heterozygote with black, *E/Ee*, *e* is not shown, because of the presence of two *E*'s against one *e*. In the heterozygote with red, *e/Ee*, the mosaic character appears, because of the presence of two *e*'s against one *E*. The homozygote *Ee/Ee* is also mosaic, but in this the extension of the red area is probably more limited than in *e/Ee*. It is known that the Japanese pattern is highly variable, and it may appear almost like pure red in some cases. It is possible that such a Japanese has the formula *e/Ee*.

As for the mechanism of the bringing about such intimate relation between *E* and *e* on the same chromosome in the Japanese strain, I am inclined to postulate unequal crossing over between homologous chromosomes, one bearing *E* and the other bearing *e* which took place once many years ago. Since *E* and *e* are allelic, this seems to be the most plausible explanation. In the heterozygous state, *E/e*, *E* shows complete dominance over *e*. In the new relation on the same chromosome, *Ee/*, the dominance is no longer realized. Thus this perhaps represents another case of position effect. The many unexpected results encountered in breeding experiments on the Japanese rabbit,[3, 4, 5] as also those on the mosaic breed of guinea-pig,[6] seem to be understandable by assuming such an unusual interrelation between the genes concerned.

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June 18, 1951

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EFFECTS OF TEMPERATURE AND HOST DENSITY ON THE RATE OF INCREASE OF AN INSECT PARASITE[1]

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Given an unlimited supply of food and a suitable environment, a population of any insect species tends to increase in an exponential manner. In nature, this unlimited growth is prevented by a series of factors that reduce the reproductive rate of the adults and destroy many of the progeny in all stages of their development. A change in the density, that is, the number of individuals per unit volume or area, of the species must result from changes in the intensity of the various mortality factors acting on the species. If these factors are to regulate the density of the insect, they must destroy proportionately more individuals when the density is high than they do when the density is low. These mortality factors include weather, nutrition, intraspecies and interspecies competition, disease, parasites, and predators. By a great oversimplification these factors may be classified into two groups: the physical, and the biotic factors. An extended discussion of these aspects of population ecology, along with extensive bibliographies, may be found in Nicholson and Bailey (1935), Smith (1935), Thompson (1939), and Solomon (1949).

The manner in which a biotic agency exerts its influence in restricting the growth of an insect population is of great practical and theoretical interest. The role that biotic agencies have in population dynamics is well typified by the way in which insect parasites act as mortality factors. The number of hosts destroyed by a parasite and the increase in parasite numbers are closely related to the number of hosts found and attacked. The ability of parasites to contact hosts, or the searching capacity of the parasites, is dependent, among other things, on the density of the hosts. Hence, the mortality caused by insect parasites is controlled, in part, by the density of the host and it is, therefore, said to be a density-dependent factor.

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In addition to destroying individual insects through direct action, physical factors modify the intensity of the action of biotic agencies acting on these insects. Changes in temperature, for example, affect the reproduction of an insect parasite, and the resulting mortality of its host, in two ways. These changes affect the rate at which insect parasites develop and reproduce. They also modify the behavior of the adult female parasites so that their ability to contact hosts when the host density is constant is affected.

The investigation of the mode of action of biotic factors in nature is extremely difficult because of the large number of factors involved and because of the complex inter-relations among them. One approach to this problem is to study one factor, or group of related factors, in the laboratory under simplified conditions. By use of a sedentary form, such as the cocoon of the European pine sawfly, the host species can be maintained at any desired density. The use of chalcid parasites, which are very small, makes it possible to construct a universe, suitable for laboratory investigations, in which the parasite population is unable to find all the hosts that it is potentially capable of parasitizing. In this case, the effect of variations in temperature, host density, and parasite density on the mortality of the host population and on the increase of the parasite population can be examined.

Several species of the sawflies belonging to the family Diprionidae comprise a group of primary importance as defoliators of conifers throughout Canada. *Neodiprion sertifer* (Geoff.), the host used in the present study, is confined to southwestern Ontario, where it is a serious pest of Scotch pine, *Pinus sylvestris* L. As part of the biological control program of the Canada Department of Agriculture against the sawfly pests of conifers, the chalcid parasite *Dahlbominus fuscipennis* (Zett.) has been imported from Europe and hundreds of millions of individuals have been propagated and released in infested areas. It has become established in most of the regions of sawfly infestation.

In this paper, the effects of variations in host density and in temperature on the ability of *D. fuscipennis* to parasitize the cocoon of one of its hosts, *N. sertifer*, are examined. The effect of variations in these two factors on the rate of oviposition of the parasite is also examined.

Ullyett (1936a) investigated the selection by *D. fuscipennis* of hosts (sawfly cocoons) distributed at different densities. He showed (1936b) that the parasite discriminates to some extent between parasitized and unparasitized hosts as well as between true and false hosts. The effect of the density of a host species on the chance of the chalcid parasite *Trichogramma evanescens* Westw. finding its host has been investigated by Flanders (1935) and Laing (1938). Varley (1941) studied the effect of host density on the egg distribution of five chalcid parasites of the knapweed gall fly and (1947) the role of these parasites as density-dependent mortality factors. Ullyett (1947) examined the relationship of mortality factors, including insect parasites, to host density in a study of natural populations of *Plutella maculipennis* (Curt.). The abilities of three species of predaceous larvae to find their host, the citrus red mite, in a uniform environment, were determined by Fleschner (1950). The effect of variations in the density of a host population on the number of hosts parasitized was analyzed in the laboratory by DeBach and Smith (1941), using puparia of the house fly and the parasites *Nasonia vitripennis* (Walk.) and *Muscidifurax raptor* Gir. and Sand. They found that the higher the host density the greater the number of hosts parasitized by these parasites. However, as the host population was increased the rate of increase in one generation was less than proportional to host density.

MATERIALS AND METHODS

Cocoons of the European pine sawfly *N. sertifer* were used as hosts, and the chalcid *D. fuscipennis* was used as the parasite. The female parasite pierces the cocoon with her ovipositor and lays her eggs on the mature larva inside. Usually, a batch of eggs is deposited during one period of oviposition and about 30 individual parasite larvae can mature on a single host. Most individual females do not attack more than two hosts.

The host cocoons were collected in the field and held in cold storage up to six months before use. To prevent the mature host larvae from destroying the parasite eggs laid on them, the hosts were paralyzed, by dipping them in hot water, before being used. The parasites used in the experiments were reared on *N. sertifer* at a temperature of 22° C. The adult female parasites were held (along with males) with a supply of water for 24–48 hours at this temperature before being used in the experiments.

The experimental universes, one of which is illustrated in figure 1, were thin, square cages of various areas. A sheet of glass, on which was placed a sheet of white blotting paper saturated with water, formed the bottom of the cage. The side, one and one-quarter inches thick, was made of three layers. A rubber gasket rested on the wet blotting paper. Attached to the gasket was a wooden frame in which there were holes, covered with organdy, for ventilation. A thin layer of felt cloth was fastened to the top of the wooden frame. A sheet of glass, resting on the felt on the four sides, formed the top of the cage. The areas of the seven cages used were 16, 25, 50, 100, 200, 300, and 400 square inches.

Each experiment was begun by placing the side of the cage on the wet blotting paper covering the bottom sheet of glass. The bottom of the cage was filled up to the ventilation holes with fresh moss, uniformly dampened by running it through a hand wringer. The upper surface of the moss was smoothed by hand. Twenty-five host cocoons were uniformly distributed, in a 5-by-5 pattern, over the surface of the moss. Twenty adult female parasites were placed near the mid-point of the moss surface but not on a host cocoon. The glass top of the cage was put in position and the cage placed in a dark incubator. The saturation deficiency of the incubator was about 6, but inside the cages it was much lower. At the end of 24 hours, the cocoons were dissected and the number of parasite eggs that each contained was recorded.

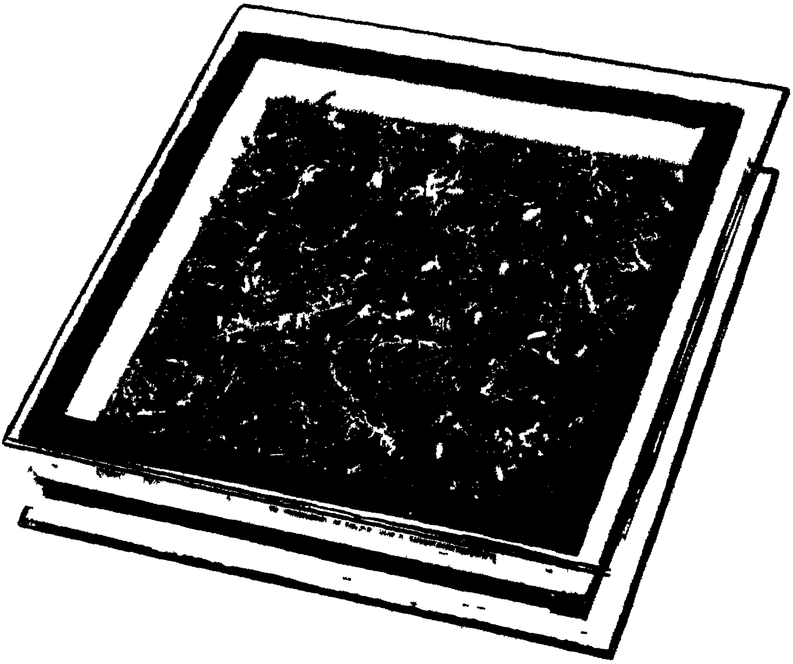

FIGURE 1. Experimental cage used in examination of the effect of host density and temperature on parasitism by *D. fuscipennis.*

Experiments at the seven different densities, that is, 0.06, 0.08, 0.12, 0.25, 0.50, 1.00, and 1.56 cocoons per square inch, were conducted simultaneously to minimize experimental error. Each experiment was replicated 15 times at temperatures of 16, 20, and 24°C.

During the development of the technique used in these investigations a number of variables, in addition to host density and temperature, that affect the number of hosts parasitized and the number of parasite eggs laid became apparent. More hosts were attacked and more eggs were laid: (1) when the adult female parasites were allowed to search for hosts in the light, (2) when the surface of the moss was smooth, (3) when the moss was dry, (4) when the host cocoons were exceptionally large, and (5) when the adult parasites were exceptionally large. Host cocoons completely covered with moss were not attacked.

PARASITISM OF THE HOST

The mean numbers of hosts parasitized by 20 female chalcid parasites when searching at temperatures of 16, 20, and 24°C. for 25 sawfly cocoons,

TABLE 1

NUMBERS OF NEODIPRION SERTIFER PARASITIZED, AT SEVEN HOST DENSITIES AND THREE TEMPERATURES, BY 20 FEMALES OF DAHLBOMINUS FUSCIPENNIS WHEN SEARCHING FOR 25 HOSTS

Host density (cocoons per square inch)	16°C.	20° C.	24°C.
	Mean	Mean	Mean
0.06	1.00 ± 0.195	2.20 ± 0.341	4.00 ± 0.543
0.08	1.33 ± 0.332	4.27 ± 0.573	5.33 ± 0.493
0.12	2.47 ± 0.350	6.47 ± 0.434	7.47 ± 0.486
0.25	3.40 ± 0.542	9.20 ± 0.554	11.20 ± 0.698
0.50	4.60 ± 0.592	12.26 ± 0.650	14.07 ± 0.733
1.00	6.80 ± 0.626	14.33 ± 0.606	16.33 ± 0.843
1.56	7.60 ± 0.616	16.27 ± 0.807	18.20 ± 0.608

uniformly arranged at seven densities, are given in table 1 and shown in figure 2. Table 1 also gives the standard error for each sample of 15 replicates. Table 1 and figure 2 show that with increase in host density the number of hosts attacked by the parasites increased rapidly at the lower host densities but tended to increase more slowly at the higher densities. In no experiment did the 20 parasites attack the 25 hosts. The results

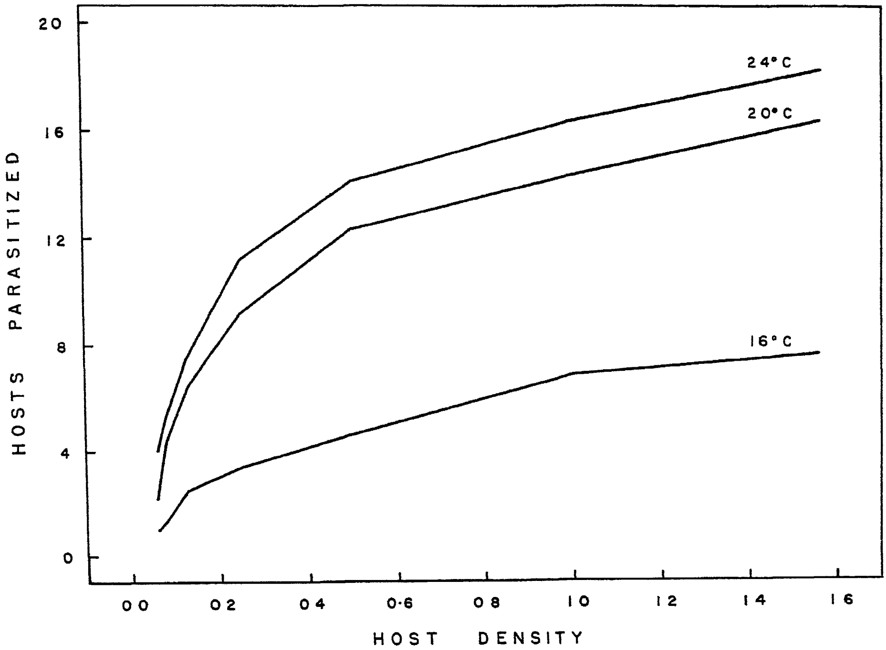

FIGURE 2. The effect of host density (cocoons per square inch) on the numbers of *N. sertifer* parasitized by *D. fuscipennis* at 16, 20, and 24 °C.

TABLE 2

NUMBERS OF EGGS LAID, AT SEVEN HOST DENSITIES AND THREE TEMPERATURES, BY 20 FEMALES OF DAHLBOMINUS FUSCIPENNIS WHEN SEARCHING FOR 25 HOSTS

Host density (cocoons per square inch)	16°C.	20°C.	24°C.
	Mean	Mean	Mean
0.06	20.00 ± 4.754	38.47 ± 7.017	88.00 ± 11.385
0.08	25.33 ± 7.131	85.27 ± 13.011	102.87 ± 12.265
0.12	40.80 ± 8.291	118.67 ± 15.224	158.47 ± 13.345
0.25	62.60 ± 9.169	184.00 ± 15.050	243.40 ± 21.410
0.50	98.20 ± 12.874	233.13 ± 16.165	293.93 ± 22.860
1.00	113.07 ± 13.381	287.73 ± 16.102	343.86 ± 22.200
1.56	136.33 ± 14.290	319.86 ± 26.575	402,00 ± 16.490

obtained at 24 and 20°C. were similar to one another except that at ~~20°~~ 16 C. fewer hosts were attacked. At the highest host density the difference between the numbers of hosts parasitized at these two temperatures was not significant (P>0.05). At 16°C. the number of cocoons attacked was much less, at comparable densities, than the number attacked at 20°C. At the lower densities the increase in the number of hosts parasitized at 16°C. was less rapid than at the higher temperatures. There was considerable variation within each series of replicates. Table 1 indicates that the standard error, in relation to the size of the mean, was much larger when

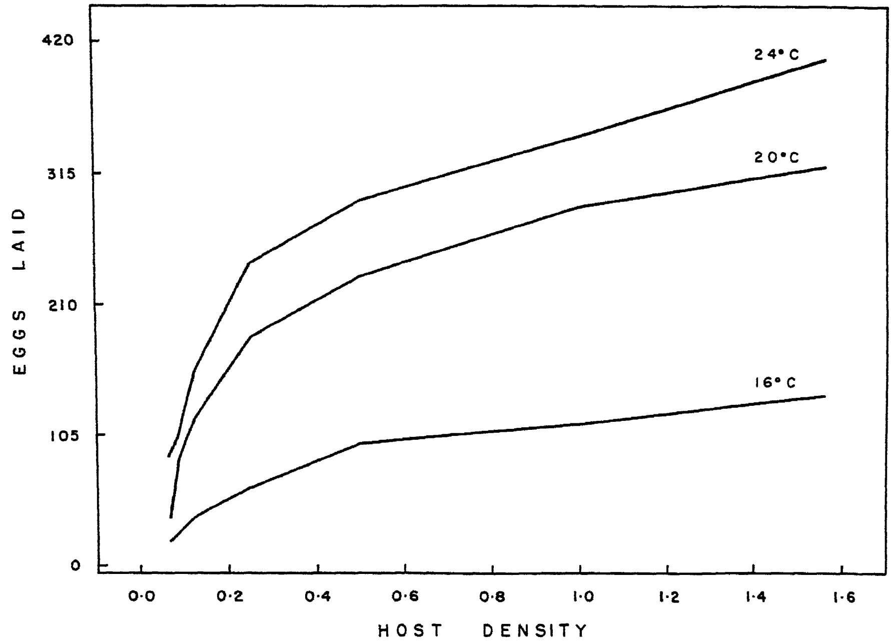

FIGURE 3. The effect of host density (cocoons per square inch) on the numbers of eggs laid by *D. fuscipennis* at 16, 20, and 24°C.

the parasites searched at the lower temperatures for hosts distributed at the lower densities. This was caused by the fact that, at the lower temperatures and densities used, the sample populations of the parasite were approaching the limits of temperature and host density at which they were able to contact hosts.

The average numbers of eggs laid by 20 female parasites at seven host densities and three temperatures are given in table 2 and shown in figure 3. A comparison of figures 2 and 3 shows that the number of eggs laid was relatively the same as the number of cocoons parasitized. At the highest host density the difference between the numbers of eggs laid at 24 and 20° C. was barely significant ($P<0.02$). At the lower temperatures and lower host densities, the variation within each set of 15 replicates was even greater than for the results given in table 1. This increase in variation can be attributed to the great variability usually found in insect oviposition.

TABLE 3

NUMBERS OF EGGS LAID, AT SEVEN HOST DENSITIES AND THREE TEMPERATURES, ON THE HOSTS PARASITIZED BY DAHLBOMINUS FUSCIPENNIS

Host density (cocoons per square inch)	16°C.	20°C.	24°C.
	Mean	Mean	Mean
0.06	21.45 ± 4.382	18.38 ± 2.919	25.07 ± 3.257
0.08	19.67 ± 2.678	19.65 ± 1.454	18.71 ± 1.341
0.12	16.30 ± 1.706	17.97 ± 1.555	20.94 ± 1.258
0.25	20.37 ± 2.744	20.28 ± 1.460	22.03 ± 1.832
0.50	21.67 ± 1.765	19.74 ± 0.957	22.04 ± 1.572
1.00	16.50 ± 1.081	20.74 ± 1.134	20.75 ± 1.016
1.56	17.89 ± 1.060	19.20 ± 1.103	22.02 ± 0.907

In table 3, the average numbers of eggs laid in each parasitized cocoon for the experiments at each density and temperature are given. Although many of the differences between densities at the same temperature and between temperatures at the same density are either not significant or just significant, at 20°C. the mean numbers of eggs per parasitized host tend to be lower than those for 24°C. and at 16°C. the means tend to be equal or lower than at 20°C. As would be expected when fewer cocoons are attacked, the standard error was much larger at the lower densities and temperatures.

In general, the number of cocoons attacked and the number of progeny left by a constant number of parasites increased rapidly with increase of host density, at low densities, but tended to level off with further increase in density. At much higher densities (unrecorded data), however, the values decreased, probably through interference of the females with each other while ovipositing. At a host density of 7.9 cocoons per square inch and a temperature of 24°C. the average number of cocoons parasitized by 20 parasites was 10.9 and the number of eggs laid was 217. At temperatures of 24 and 20° C. the relations of parasite reproduction to host density were relatively the same, but at 16° C. there was a marked reduction in the numbers of hosts parasitized and the numbers of eggs laid. The numbers of eggs laid on a host at the different densities and temperatures were nearly equal but were slightly reduced as the temperature was lowered. There was a great deal of variation within the replicates, particularly at the lower densities and temperatures.

EFFECT OF VARIATION IN AREA OF UNIVERSE

In the experiments reported in tables 1, 2, and 3 there were two controlled variables: host density and the area of the surface over which the parasites searched for hosts. Possibly the variation in parasitism was related to the area of the universe rather than host density. To eliminate the effect of variation in area, the area of the experimental cages was kept constant at 50 square inches and the number of hosts was varied from 2 to 64, giving the following densities of hosts: 0.04, 0.08, 0.18, 0.32, 0.50, 0.72, 1.00, and 1.28 cocoons per square inch. Twenty adult female parasites searched at 24° C. under conditions otherwise identical with those prevailing in the earlier experiments. The experiments were replicated 15 times.

For the experiments at 24°C., data concerning the number of cocoons parasitized, the numbers of eggs laid, and the numbers of eggs per parasitized cocoon are given in table 4. Figure 4 shows the numbers of cocoons attacked and figure 5 the numbers of eggs laid by the 20 females at the eight different host densities.

TABLE 4

PARASITISM, AT 24° C., OF NEODIPRION SERTIFER BY DAHLBOMINUS FUSCIPENNIS WHEN THE NUMBER OF HOSTS IS VARIED AND THE AREA OF SEARCH IS CONSTANT

Host density (cocoons per square inch)	Cocoons parasitized	No. of eggs laid	No. of eggs per parasitized cocoon
	Mean	Mean	Mean
0.04	1.80 ± 0.144	84.07 ± 28.144	48.53 ± 5.180
0.08	3.13 ± 0.134	143.87 ± 13.565	44.88 ± 3.697
0.18	5.73 ± 0.371	197.00 ± 16.334	35.02 ± 2.572
0.32	9.80 ± 0.470	268.20 ± 18.218	27.23 ± 0.993
0.50	12.93 ± 0.808	296.67 ± 22.228	23.00 ± 1.269
0.72	15.63 ± 0.859	344.93 ± 26.779	22,62 ± 1.214
1.00	17.53 ± 0.833	349.27 ± 17.054	20.14 ± 0.941
1.28	18.40 ± 0:989	361.73 ± 19.521	19.97 ± 0.909

The numbers of cocoons attacked were similar, at comparable densities, to those recorded at 24° C. in table 1. At the lower densities (figure 4) the curve is more nearly linear and this is related to the fact that there was a limited number of cocoons available for attack. The small number of cocoons caused a marked reduction in the standard error.

The average numbers of eggs laid per 20 female parasites were similar, at comparable densities, to those recorded in table 2. There were slightly more eggs laid at the lower densities but about the same number at the higher densities. The variation was much the same.

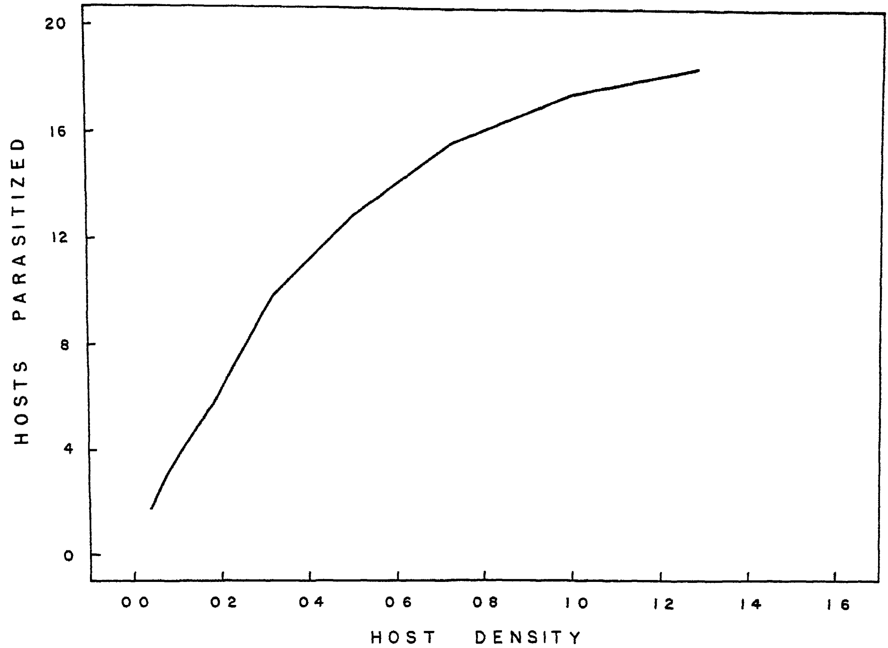

FIGURE 4. The numbers of hosts parasitized at 24°C. when the area of search was constant and the number of hosts varied.

The small number of cocoons used at the lower densities resulted in a large number of eggs being deposited in individual cocoons. This is an indication that there was a considerable amount of superparasitism. The results at the higher densities were similar to those recorded in table 3.

In general, varying the host density by changing the area of search or the number of hosts available did not affect the relationship between the host density and the number of hosts parasitized or the number of eggs laid. Therefore, it appears that the actual, in contrast with the potential, reproductive rate of the parasite varies with the density of the host.

EFFECT OF TEMPERATURE ON OVIPOSITION

The parasites used in the experiments reported in tables 1, 2, and 3 were reared at 22°C. and held after emergence for 24–48 hours at the same temperature. They were then used at temperatures of 16, 20, and 24°C. for a period of 24 hours. Thus, the effect of the variation in temperature in the experimental cages on the oviposition of the parasites was minimized. However, it is possible that the results obtained at the highest density were determined by the effect of temperature on the rate of oviposition of the parasite and not by the effect of temperature on the ability of the parasite to contact the host. To investigate this possibility 100 female parasites were enclosed individually in small glass vials containing two host cocoons. The 100 vials were placed inside an experimental cage, and the cage was placed in an incubator. In this manner, the oviposition of 100 individual females exposed to the same saturation deficiency and temperatures as previously used was recorded.

In table 5 the results of the oviposition by the 100 female parasites are given. With a decrease in temperature from 24 to 16° C. the number of females that failed to oviposit rose from 11 to 25 and the number of eggs laid dropped from 1993 to 1363. However, the number of eggs laid by each female that oviposited decreased by only four. At 16° C. fewer females attacked two hosts. When the parasite attacked two cocoons, one host received more eggs than the other. At 24° C. the cocoons that received the greater number of eggs, in each of the 20 pairs attacked, accounted for 65 per cent of the total eggs laid on those 20 pairs of hosts. At 20° C., for 20 pairs, and at 16° C., for 6 pairs, the percentages were 71 and 76 respectively.

The effect of temperature on the oviposition of the 20 parasites in the experimental cages can be estimated by comparing the results obtained at the highest host-density with one-fifth of those obtained for the 100 parasites in the glass vials. This is done in table 5, in which the observed values for 20 female parasites are taken from tables 1, 2, and 3, and the calculated values are derived from the left-hand part of table 5.

In the first row of table 5, the number of eggs laid by 20 parasites in the experimental cages is seen to approach the number that 20 females are able to lay at 20 and 24° C. but to be only half the possible number at 16° C. In the second row, the numbers of cocoons parasitized in the cages

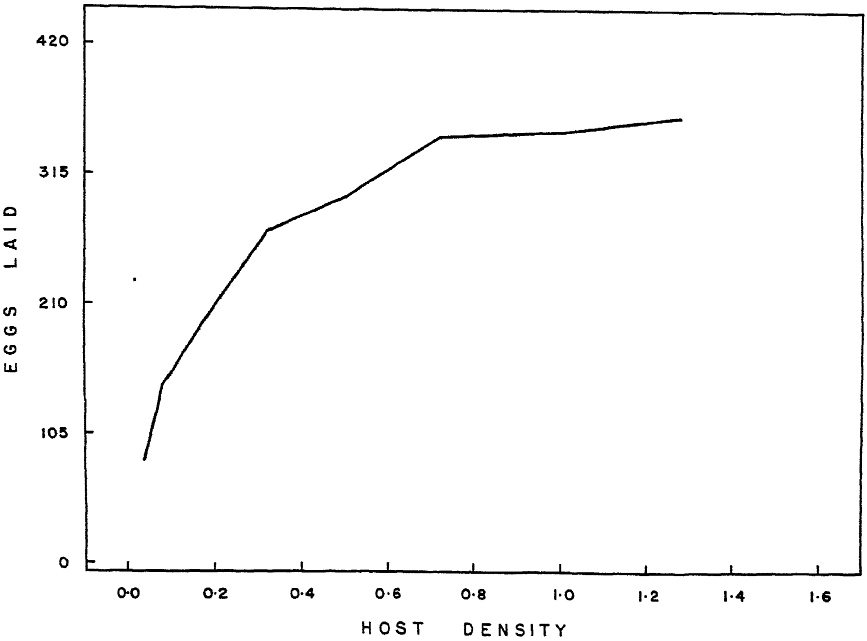

FIGURE 5. The numbers of eggs laid by *D. fuscipennis* at 24° C. when the area of search was constant and the number of hosts varied.

at 20 and 24° C. were equal to the numbers of females that could oviposit but at 16° C. the number was again half that of the potential number. However, at each of the three temperatures the number of eggs laid in each parasitized cocoon was almost identical with the number of eggs that a single female was able to lay in a 24-hour period. Though the number of cocoons parasitized varied from 7 to 18, out of a possible 25, each cocoon parasitized received on the average all the eggs a female was capable of laying. Therefore, although in the glass vials 20 females parasitized an average of 21.8 cocoons (109/5) at 24° C., 21.2 cocoons at 20° C., and 16.2 cocoons at 16° C., in the experimental cages each female probably attacked on the average only one host. In this case, the low fecundity of the parasite at 16° C. was caused by the fact that it parasitized only half of the number that it is capable of parasitizing.

TABLE 5

EFFECT OF TEMPERATURE ON OVIPOSITION OF DAHLBOMINUS FUSCIPENNIS

	100 Females (1 per vial)			20 Females					
	16° C.	20° C.	24° C.	16° C.		20° C.		24° C.	
				Cal.	Obs.	Cal.	Obs.	Cal.	Obs.
Eggs laid	1363	1662	1993	272.60	136.33	332.40	319.86	398.60	402.00
Females ovipositing	75	85	89	15.00		17.00		17.80	
Cocoons parasitized				16.2	7.60	21.2	16.27	21.8	18.20
Eggs per ovipositing female	18.17	19.55	22.39	18.17		19.55		22.39	
Eggs per parasitized cocoon					17.89		19.20		22.02
Females parasitizing one cocoon	69	66	69						
Females parasitizing two cocoons	6	20	20						

In general, at the highest host density, the results obtained at 20 and 24° C. can be attributed to the effect of temperature on the ability of the parasite to lay eggs. Since the curves in figures 1 and 2 are similar in form for the two temperatures, the effect was apparently uniform at the various densities. At 16° C. the results obtained at the highest host density were only half as large as possible. It seems in this case that temperature not only reduced the capacity of the parasite for oviposition but also acted in conjunction with host density to reduce the number of hosts contacted by the parasite.

PARASITIZATION OF THE HOST IN RELATION TO HOST DENSITY

The relation between host density and the increase in a laboratory population of a parasite has been found by Gause (1934) to be curvilinear and to approximate the curve

$$y = a(1 - e^{-kx})$$

where y = number of hosts attacked, x = density of the hosts, a = the limit approached asymptotically by y, e = the base of natural logarithms, and k = a constant related to the rate of change of y. This curve is based on the assumption that the parasite contacts hosts at random. DeBach and Smith (1941) found that this curve approximately described the results obtained, in a single generation, with one insect parasite but not those obtained with a second species. Ullyett (1947) concluded that the host density and parasitism of a natural insect population were related by the function

$$y = ax^b$$

where y = host larvae per host plant, x = parasites per plant, and a and b are constants. Neither of these two formulae gave a sufficiently close approximation to the results observed in this study.

TABLE 6

PARASITIZATION OF THE HOST IN RELATION TO HOST DENSITY

Host density (cocoons per square inch)	Hosts parasitized				Parasite eggs laid			
	16°C.		24°C.		16°C.		24°C.	
	Obs.	Cal.	Obs.	Cal.	Obs.	Cal.	Obs.	Cal.
0.06	1.00	0.72	4.00	4.32	20.00	16.42	88.00	88.28
0.08	1.33	1.37	5.33	5.61	25.33	27.01	102.87	106.45
0.12	2.47	2.29	7.47	7.42	40.80	41.93	158.47	156.15
0.25	3.40	3.83	11.20	10.49	62.60	67.15	243.40	223.24
0.50	4.60	5.29	14.07	13.55	98.20	92.37	293.93	290.34
1.00	6.80	6.56	16.33	16.61	113.07	117.63	343.86	357.53
1.56	7.60	7.19	18.20	18.58	136.33	133.79	402.00	400.52
Chi square		0.2414		0.1057		1.8166		2.5485
d.f.		3		5		6		6
P		> .95		> .99		> .90		> .80

Ullyett (1936a) tested the assumptions that the area searched by individual females of *Dahlbominus fuscipennis* varied inversely as the host density and that the searching was at random. He concluded that climate had no effect on the results obtained.

In the data of this study, it was observed by E. C. Pielou that the rate of change of parasitization of the host varied as the inverse of host density and that consequently the relationship between parasitism and host density might be expressed by

$$y = a + b \ln x$$

where y = no. of hosts attacked, or number of parasite eggs laid, ln x = natural logarithm of host density, and a and b are constants.

TABLE 7

RELATIVE NUMBERS OF HOSTS AND PARASITES EMERGING AT THE END OF A SINGLE GENERATION OF THE PARASITE

Host density (cocoons per square inch)	16°C.			20°C.			24°C.		
	Hosts emerging	Parasites emerging	Ratio parasites/ hosts	Hosts emerging	Parasites emerging	Ratio parasites/ hosts	Hosts emerging	Parasites emerging	Ratio parasites/ hosts
0.06	24.00	20.00	0.8	22.80	38.47	1.7	21.00	88.00	4.2
0.08	23.67	25.33	1.1	20.73	85.27	4.1	19.67	102.87	5.2
0.12	22.53	40.80	1.8	18.53	118.67	6.4	17.53	158.47	9.0
0.25	21.60	62.60	2.9	15.80	184.00	11.6	13.80	243.40	17.6
0.50	20.40	98.20	4.8	12.74	233.13	18.3	10.93	293.93	26.9
1.00	18.20	113.07	6.2	10.67	287.73	26.9	8.67	343.86	39.7
1.56	17.40	136.33	7.8	8.73	319.86	36.6	6.80	402.00	59.1

In table 6, the numbers of hosts parasitized and the numbers of eggs laid at 16 and 24°C. are compared with the numbers expected on the basis that the increase in the rate of parasitization of the host varies with the inverse of host density. In all cases, there is close agreement between observed and calculated values. Table 6 indicates that the effect of host density on the parasitization of the host by *D. fuscipennis* is uniform, in principle, within the normal temperature range of the parasite. The absolute values obtained and the rate at which the effect changes, however, are different at different temperatures.

EMERGENCE OF HOSTS AND PARASITES

Some indication of the effects of temperature and host density on the efficiency of the parasite can be obtained by comparing the relative emergence of the host and that of the parasite adults at the end of a *single generation* of the parasite. In the present case, 20 female parasites were searching for 25 hosts at seven different densities and three different temperatures. It is assumed that all unparasitized hosts emerged as adults, although, of course, this would not occur under natural conditions. Likewise, it is assumed that all parasite eggs developed into adult parasites, for it is known that at least 30 parasite progeny will develop on a single host.

In table 7 the number of hosts, out of the 25 originally exposed to the parasites, that would emerge as adults is compared with the number of parasites that would emerge as adults (taken from table 2) when a parasite generation is taken to be 24 hours. The ratio of parasites to hosts is calculated for each of the three temperatures. As to be expected from table 1, with increase in host density and temperature there was a decline in the number of hosts emerging. The rate of increase of the parasite was less than proportional to the increase in host density, except in the range 0.06 to 0.12 cocoons per square inch, where it was about equal. The ratio of parasites to hosts emerging at the end of a single parasite generation increased with the density of the host and with temperature. At 16 and at 24°C. the increase was as great as tenfold with increase in host density. At 20°C. the increase was greater but the values for the low densities appeared to be a little low. At comparable densities, there was a fourfold increase, between 16 and 20°C., in the ratio of emerging parasites to emerging hosts, while between 16 and 24°C. there was a fivefold increase. In general, the increase of the ratio was approximately proportional to the increase of host density up to 0.25 cocoons per square inch and then it became less.

DISCUSSION

In this laboratory study, the number of hosts destroyed by the parasite *D. fuscipennis* and the increase, in a single generation, of the parasite population were controlled by the two variables: temperature and host density. Any increase in the density of the host caused an increase in the mortality of the host and brought about an increase in the parasite population. But the rate at which these increases occurred decreased with increments in host density. The operation of this process was greatly influenced by variation in one physical factor, temperature. In the field, this process, though no doubt differing in the magnitude of its effect, provides one check on the unlimited increase of the population of an insect pest. This study indicates that the parasite was least efficient, as a mortality factor, at the lower host densities and temperatures. Since these most nearly approach the conditions found in the field, it seems likely that the parasite attacks far fewer hosts than it is capable of attacking. On the other hand, under these circumstances an increase in host density or temperature produces the greatest response of the parasite as a mortality factor. Through the use of the sedentary host, the sawfly cocoon, and the chalcid parasite *D. fuscipennis*, this aspect of the problem can be examined under simplified field conditions.

SUMMARY

1. The searching of the chalcid parasite *Dahlbominus fuscipennis* (Zett.) for its host *Neodiprion sertifer* (Geoff.) was examined in the laboratory at temperatures of 16, 20, and 24° C. when the host was uniformly distributed at seven densities ranging from 0.06 to 1.56 cocoons per square inch.
2. At lower host densities, the rate of increase of the parasite was rapid, but at the higher host densities it tended to level off.
3. Variations in area of search and in number of hosts available for attack did not account for the variation in parasite increase.
4. At 24 and 20° C. the rate of increase of the parasite, at the highest host density, was controlled by the effect of temperature on the parasite's oviposition but at 16° C. only half as many hosts were attacked and half as many eggs were laid as was possible.
5. In a single parasite generation, the relation between parasitism and host density approximated the curve $y = a + b \ln x$.
6. Increase in temperature and in host density caused a large increase in the ratio of parasites emerging to hosts emerging in a single parasite generation.

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EVIDENCE CONCERNING THE STRUCTURE OF AN EAR OF CORN (ZEA MAYS, L.)[1]

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The maize ear, with its numerous rows of paired spikelets, is morphologically unique among crop plants. This structure has provoked considerable debate among botanists, geneticists, and morphologists concerning its phylogenetic development, its structure, and its function. The development of the ear has been investigated extensively by Collins (1912, 1919, 1925, 1931), Collins and Kempton (1920), Kempton (1919), Weatherwax (1918, 1919, 1935), Anderson (1944a, 1944b), Bonnett (1940), Mangelsdorf (1945, 1948), Cutler (1946), Fujita (1939), Laubengayer (1948), Stephens (1948), and others. An excellent review has been published by Mangelsdorf (1945) along with convincing evidence that certain characteristics of the ear are explicable on the basis of the Mangelsdorf-Reeves (1939) hypothesis of the origin of corn. He points out that the uniqueness of the maize ear is not due to any one of its morphological characteristics but rather to its combination of being wholly pistillate and having a massive rachis with large, naked caryopses arranged in even numbers of eight or more vertical rows.

A photograph is presented here of two rather unusual ears of corn which serves to show the basic anatomy of the ear. With arguments based on this material I have taken the liberty of speculating about the phylogenetic development of the maize ear.

MATERIALS

The two ears shown in figure 1 came from different hybrid populations. The four-rowed ear developed at the lowermost node of the plant from an F_1 population of Hickory King and a four-rowed sweet variety. The eight-rowed ear appeared in an F_2 population of an eight-rowed yellow starchy line with the four-rowed sweet variety. These two populations were planted in a greenhouse experiment along with others on the 5th of January, 1947, using 2½ inch pots, and later planted to 6-inch pots. A photoperiod of daily decreasing length was provided for the first twenty days and thereafter with the seasonal increasing photoperiod. As the plants began to tassel the tassels were removed as soon as they started shedding pollen. By limiting the amount of pollination the lower ears were induced to develop.

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EAR MORPHOLOGY

During the routine examination of about 5,000 immature ears from the greenhouse material, in connection with a study of inheritance of kernel row number, several were found which, from a morphological point of view, were highly interesting. Two of these ears are shown in figure 1.

We may picture, as Collins (1919) and others have done, the phylogenetic development of the maize ear as requiring at least three major steps in addition to the development of the monoecious habit. The progenitor is conceived as being two-ranked, single-flowered, and alternate. These steps, although not necessarily in this chronological order, are the development of the double flowered alicoles, the shortening or compacting of the internode to form the massive rachis and bring the spikelets together in a compact, two-ranked spike, and the elaboration of this two-ranked spike by fusion or twisting to form the many-ranked spike of modern maize.

The elongation at the tip of the four-rowed ear (figure 1), as well as the alternate arrangement of the pairs of kernels in the lower part of the ear, attest to the condensation by "yoking" postulated by Collins (1919). If the elongation evident at the tip were complete, this ear would obviously be an alternate two-ranked spike with double-flowered alicoles.

The basal portion of the eight-rowed ear shows rather well the spiral phyllotaxy, or what has been sometimes called the false spiral phyllotaxy, which may be traced in most normal ears of corn. However, the elongate tip of this ear shows not the spiral phyllotaxy but a whorled one in which the internodes are quite distinct. This may be a small point because the distinction between spiral and whorled phyllotaxy is not always clear, but it does appear reasonable that if the two-ranked spike twisted to produce the four-ranked spike, it would leave a basic spiral type of phyllotaxy, not the whorled one shown here. If, however, each node in the prototype produced two alicoles instead of one, then this, in combination with the "yoking" evident in the four-rowed ear, would be the basis for an eight-rowed ear. This is essentially in agreement with the fusion idea of the elaboration of the two-ranked spike. Corn from the Guarany Indians of Paraguay in crosses with pod corn quite regularly produces these elongate tips showing, definitely, a whorled phyllotaxy (Mangelsdorf, 1945, 1948). Anderson and Brown (1948) have also pointed out that the basic phyllotaxy of the ear and tassel appears to be whorled.

Evidence considered by Stevens (1948) led him to agree, essentially, with Collins' (1919) postulate, namely, that node condensation or telescoping is the basis of the polystichous spike of maize. The evidence presented here cannot be interpreted to differ with this hypothesis. However, the evidence up to now suggests, at least to this writer, that condensation occurred first in a two-ranked spike and was followed by further elaboration at each node.

A word of caution is appropriate in connection with phylogenetic inferences which might be drawn from these ears. If what we observe in North American maize is the result of an undetermined amount of later tripsacoid

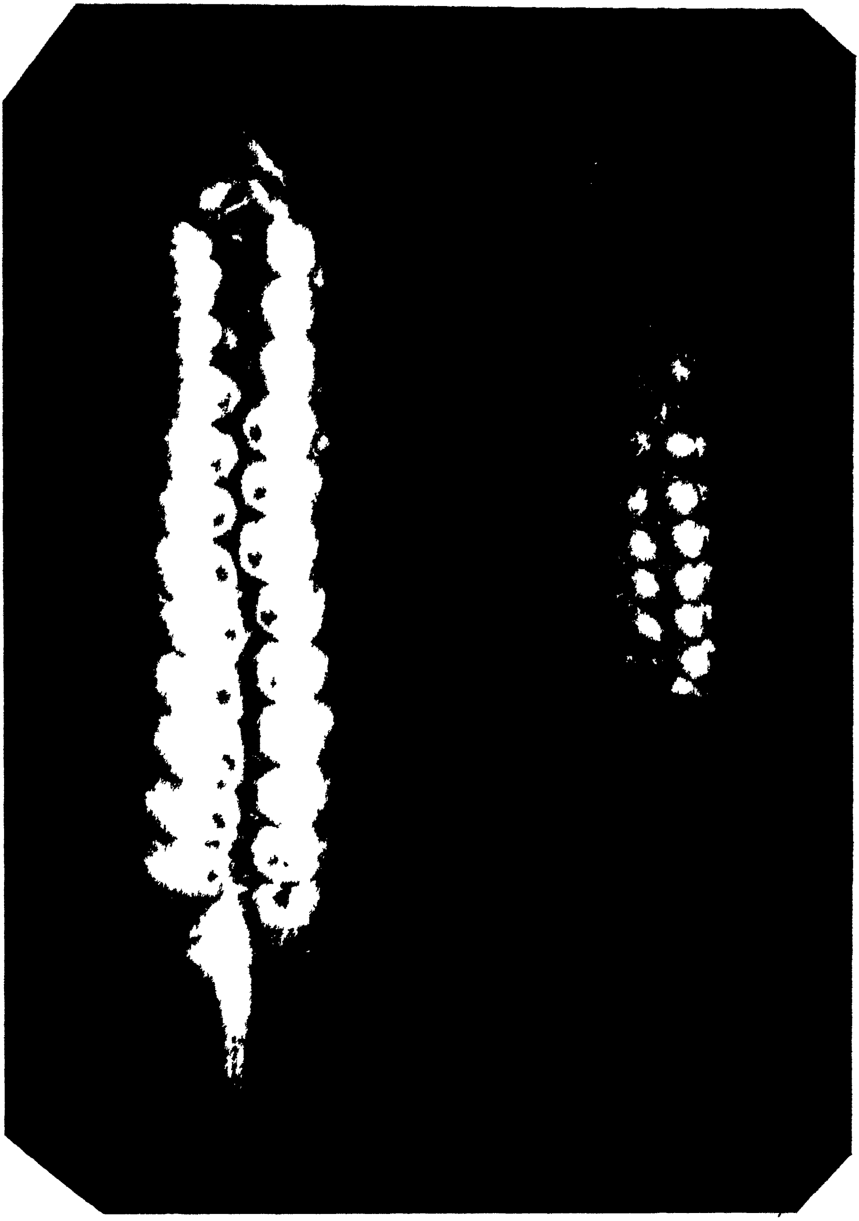

FIGURE 1 Immature four- (left) and eight-rowed (right) ears of corn, showing the pattern of fasciation.

influence, then our conclusions concerning the structure of the ear are somewhat less valid. Considering further that most authorities agree North America is not the most likely point of origin of maize it should follow that evidences from North American maize are drawn from "...a highly derived rather than a primitive condition in Zea maize" (Anderson, 1944). The Guarany material of Mangelsdorf is considered a primitive type; at least it has not suffered the same contamination as has the North American form.

SUMMARY

Evidence is presented which is interpreted as support for the fusion hypothesis of the origin of the maize ear. The basic phyllotaxy of the maize ear appears to be whorled.

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THE RANDOM DISTRIBUTION OF GENES ON CHROMOSOMES

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It is sometimes of interest to know the probable distribution on the chromosomes of a species of the genes affecting a character or characters. As an example, in crosses made for the purpose of combining desirable characteristics of the parent stocks, the ease with which the desired recombinations can be obtained depends, among other things, on the amount of linkage among the genes determining the characteristics. Knowledge of the most probable distribution of the genes concerned gives some idea of the amount of linkage to be expected. As another example, one might wish to know the probability that, of a known number of genes affecting a character, a given number will be located on a single chromosome

If the chromosomes are approximately equal in size and if the number of genes involved is known or can be estimated, the probabilities for the various possible distributions of the genes on the chromosomes can be calculated by means of the multinomial theorem. The purpose of this paper is to exhibit the way in which this calculation can be accomplished. A general discussion of the multinomial theorem can be found in David (1949).

THE MULTINOMIAL EXPANSION

Let n be the haploid number of chromosomes and let s be the number of genes whose distribution is of interest. If the chromosomes are equal in size and if the distribution of genes on the chromosomes is random, there is equal probability that a given one of the s genes will be located on any one of the n chromosomes. This probability is 1/n. Let p_i (= 1/n) be the probability that a given gene will be located on any one of the chromosomes, where the index $i = 1, 2, \ldots, n$. Then the array of probabilities for a given gene is

$$(p_1 + p_2 + \cdots + p_n).$$

The array of probabilities for any other gene is the same as for the first and the array of probabilities for two genes together is the product

$$(p_1 + p_2 + \cdots + p_n)^2.$$

For s genes the array of probabilities can be represented by the expansion of the multinomial to the s power.

Each term in the expansion of the multinomial contains a product of probabilities and a coefficient. In each term there is one p_i element from each of the s quantities $(p_1 + p_2 + \ldots + p_n)$ which are multiplied together in the expansion. Since $p_1 = p_2 = \ldots = p_n = \frac{1}{}$ this part of the term will be

$p^s = \frac{1}{n^s}$. No particular order of taking the p_i symbols is specified. The coefficient of the term, derived from elementary probability theory, gives the number of different orders in which this particular set of p_i elements can be taken.' It is given by the expression

$$\frac{s!}{(1!)^{a_1}\,(2!)^{a_2}\ldots(j!)^{a_j}},$$

where a_1 is the number of p_i elements which occur once in the term, that is, the number of chromosomes with one gene, a_2 is the number which occur twice, ..., a_j is the number which occur j times. The complete term of the multinomial expansion is then

$$\frac{s!}{(1!)^{a_1}\,(2!)^{a_2}\ldots(j!)^{a_j}}\cdot\frac{1}{n^s}. \tag{1}$$

To illustrate what this means, consider the problem of the distribution of three genes in an organism with four chromosomes in the haploid set. The chromosomes are numbered from one to four. Let p_1 be the probability that a particular gene is located on chromosome 1, and p_2, p_3, and p_4 the probabilities that it is located on chromosome 2, 3, and 4, respectively. Then $p_1 = p_2 = p_3 = p_4 = \frac{1}{n} = \frac{1}{4}$. The expansion of the multinomial $(p_1 + p_2 + p_3 + p_4)^3$ gives the probabilities for the various possible distributions of the three genes on the four chromosomes. One of the terms in the expansion is $3p_1^2p_2$ ($= {}^3\!/_{64}$) which gives the probability that two genes will be located on chromosome 1 and one on chromosome 2. Without actually expanding the multinomial we could arrive at this probability by use of (1). That is,

$$\frac{3!}{(1!)^1\,(2!)^1}\cdot\frac{1}{4^3} = \frac{3}{64}.$$

In addition to knowing the value of a single term, we might be interested in knowing the number of similar terms. We might want to know, for example, the number of terms which give probabilities for two genes on any one chromosome and one gene on any other. Such terms would be $3p_1^2p_2$, $3p_1p_2^2$, $3p_3^2p_4$, etc. We want to know how many ways we may make three selections from four chromosomes so that two of them are not taken (have no genes), one is taken once (has one gene), and one is taken twice (has two genes). This is given by the expression

$$\frac{n!}{a_0!a_1!\ldots a_j!}, \tag{2}$$

where a_0 is the number of chromosomes with no genes, a_1 the number with 1 gene, ..., a_j the number with j genes. The complete expression which gives the probability for getting a_0 chromosomes with no genes, a_1 chromosomes with 1 gene, ..., a_j chromosomes with j genes is therefore the product of (1) and (2),

$$\frac{n!}{a_0!\,a_1!\,\ldots\,a_j!}\cdot\frac{s!}{(1!)^{a_1}\,(2!)^{a_2}\ldots(j!)^{a_j}}\cdot\frac{1}{n^s}. \qquad (3)$$

In the foregoing example this would be

$$\frac{4!}{2!1!1!}\cdot\frac{3!}{(1!)^1\,(2!)^1}\cdot\frac{1}{4^3}=12\cdot\frac{3}{64}=\frac{9}{16}.$$

THE NUMBER OF CHROMOSOMES OCCUPIED BY GENES

For small values of s and n it is easy to specify all possible distributions and to calculate the probabilities of their occurrence by the use of (3). For larger values of s and n this may become tedious. If a more limited specification of the distribution is made by combining certain classes of arrangements so that the number of such classes is small, the probabilities can be easily calculated.

Suppose that instead of specifying the number of chromosomes with 0, 1, 2, ..., j genes, we specify only the number with one or more genes. Denote this number by r ($r \leq n$, $r \leq s$). Then n-r is the number with no genes. The probability that all the genes will be located on exactly r of the n chromosomes is the product of the following terms: (a) the number of ways that r chromosomes can be taken out of n chromosomes, (b) the number of ways of arranging s genes on r chromosomes, and (c) the product of probabilities $\frac{1}{n^s}$. The number of ways that r chromosomes can be taken out of n chromosomes is given by the expression

$$n\,(n-1)\,(n-2)\ldots(n-r+1)=\frac{n!}{(n-r)!}.$$

The number of ways of arranging s genes on r chromosomes is the same as the number of ways of dividing s things into r groups. This quantity is given by the expression, $\frac{\Delta^r 0^s}{r!}$, derived by use of the calculus of finite differences (Stevens, 1937). It is tabled as the "differences of zero" (Stevens, 1937) for values of r and s from 1 to 25. The same table may be found in Fisher and Yates (1943) entitled "the leading differences of powers of natural numbers."

The probability that all s genes will be located on exactly r chromosomes is, therefore,

$$\frac{n!}{(n-r)!}\cdot\frac{\Delta^r 0^s}{r!}\cdot\frac{1}{n^s}. \qquad (4)$$

TABLE 1

DISTRIBUTION OF s = 15 GENES ON n = 21 CHROMOSOMES. THE PROBABILITIES ARE COMPUTED BY (4) (SEE TEXT).

No. of chromosomes occupied by one or more genes r	$\frac{\Delta^r 0^s}{r!}$		Probability
15	1		0.00104
14	105		0.01562
13	4 550		0.08463
12	106 470		0.22004
11	1 479 478		0.30576
10	12 662 650		0.23791
9	67 128 490		0.10514
8	216 627 840		0.02609
7	408 741 333		0.00352
6	420 693 273		0.00024
5	210 766 920		0.00001
		Total	1.00000

In table 1 for s = 15 genes, n = 21 chromosomes, and for varying values of r are given the values of $\frac{\Delta^r 0^s}{r!}$ and the probabilities calculated from (4) that all genes will be located on exactly r chromosomes. A comparison of the probabilities shows that the most probable arrangement is for the fifteen genes to occupy eleven chromosomes.

A further examination of this example may help to clarify the relationship between (3) and (4). For any value of r it is possible to list the various arrangements which can occur. Thus, in our example, when r = 15 there is only one possible arrangement, one gene on each of fifteen chromosomes (1^{15}). When r = 14 there is also only one arrangement, one gene on each of

TABLE 2

THE WAYS IN WHICH s = 15 GENES CAN BE DISTRIBUTED ON r = 11 CHROMOSOMES. IN THE BODY OF THE TABLE ARE THE NUMBERS OF CHROMOSOMES HAVING THE SPECIFIED NUMBER OF GENES. THE PROBABILITIES ARE COMPUTED BY (3); THE NUMBER OF WAYS THE ARRANGEMENTS CAN OCCUR ARE COMPUTED BY (5) (SEE TEXT).

No. of genes per chromosome					Probability	No. of ways arrangement can occur
1	2	3	4	5		
10				1	0.00062	3 003
9	1		1		0.01552	75 075
9		2			0.01034	50 050
8	2	1			0.13964	675 675
7	4				0.13964	675 675
					0.30576	1 479 478*

*This number equals $\frac{\Delta^r 0^s}{r!}$ for r = 11, s = 15.

thirteen chromosomes and two genes on the remaining chromosome (1^{13}, 2^1). When $r = 13$ there are two possible arrangements (1^{12}, 3^1; 1^{11}, 2^2), and so on. The five possible arrangements when $r = 11$ are given in table 2. By use of (3) the probabilities for the occurrence of each can be calculated (next to last column, table 2).

Since $a_0 = (n-r)$, (3) can be rewritten

$$\frac{n!}{(n-r)!}\cdot\frac{s!}{a_1!(1!)^{a_1}a_2!(2!)^{a_2}\dots a_j!(j!)^{a_j}}\cdot\frac{1}{n^s}.$$

By comparison with (4) it can now be seen that

$$\frac{s!}{a_1!(1!)^{a_1}a_2!(2!)^{a_2}\dots a_j(j!)^{a_j}} \tag{5}$$

gives the number of ways in which s genes can occupy exactly r chromosomes when it is specified that a_1 chromosomes shall have 1 gene, a_2 chromosomes shall have 2 genes, ..., a_j chromosomes shall have j genes, $\frac{\Delta^r 0^s}{r!}$ gives the total number of ways in which s genes can occupy exactly r

TABLE 3

ALL ARRANGEMENTS OF $s = 10$ GENES ON $r = 20$ CHROMOSOMES IN WHICH THERE ARE 3 OR MORE GENES ON AT LEAST ONE CHROMOSOME. STARRED ARRANGEMENTS ARE THOSE WITH EXACTLY 3 GENES ON ONE CHROMOSOME AND NO MORE THAN 2 GENES ON ANY OTHER.

No. of chromosomes occupied by one or more genes r	No. of genes per chromosome							Probability
	1	2	3	4	5	6		
8	7		1				*	0.05942
7	6			1				0.00801
	5	1	1				*	0.09615
6	5				1			0.00069
	4	1		1				0.00858
	4		2					0.00572
	3	2	1				*	0.03434
5	4					1		0.00004
	3	1			1			0.00046
	3		1	1				0.00076
	2	2		1				0.00172
	2	1	2					0.00229
	1	3	1				*	0.00229
4	all arrangements have 3 or more genes on at least one chromosome							0.00039
3								0.00001
2								0.00000
1								0.00000
	Total							0.22097
	Total of starred arrangements							0.19230

chromosomes or the sum of the values calculated by (5) over all possible arrangements of s genes on exactly r chromosomes. The last column of table 3 gives the calculated number of ways for each arrangement when s = 15 and r = 11. The sum of these values is $\frac{\Delta^r 0^s}{}$

AMOUNT OF LINKAGE

In the example of the distribution of fifteen genes in a species with twenty-one chromosomes (for example, *Triticum vulgare*, in which the chromosomes are nearly equal in size) it can be seen from table 1 that the probability for getting no linkage, that is, for the fifteen genes to occupy fifteen chromosomes, is only 0.00104. It is much more likely that there will be some linkage. The most probable arrangement is that the fifteen genes will occupy only eleven chromosomes, in which case from five to eight genes will be on the same chromosome with at least one other gene. This means that there will most probably be eleven rather than fifteen independently assorting units. The closeness of linkage of those genes which are linked also, of course, affects recombination frequency, but this factor will not be considered here.

NUMBER OF GENES ON ONE CHROMOSOME

Dunn and Caspari (1945) were interested in calculating the probability that, of ten known genes affecting the tail of the mouse, three would be located on the same chromosome. There are twenty pairs of chromosomes in the mouse. They vary somewhat in size, thus failing to fulfill the assumption of equal probabilities ($p_1 = p_2 = \ldots = p_n$) for all the chromosomes. Stevens (1937) has shown that the effect of unequal probabilities is to decrease r, the number of chromosomes which carry genes, and to increase the number of genes per chromosome. The result of the following calculation, based on the assumption of equal probabilities, is therefore an underestimate of the true probability.

The question must be more exactly specified. Of a number of possible questions two are selected for illustration here. (1) What is the probability that there will be three or more genes on at least one chromosome; (2) what is the probability that there will be exactly three genes on one chromosome and no more than two genes on any of the others? The first question includes all possible arrangements having three genes on one chromosome and the answer to it gives the maximum probability for such an arrangement to occur. The second question includes only a small number of arrangements but those which probably are similar to the true arrangement in the mouse.

It is necessary to list all arrangements which meet the specified conditions. This is done in table 3. Fortunately all possible arrangements for $r \leq 4$ are included and the probabilities for the occurrence of these can be calculated from (4). For values of r from five to eight the probabilities can be calculated from (3). The sum of the probabilities for all arrangements in table 3, 0.22097, gives the probability for getting three or more genes on at least one chromosome. The sum of the probabilities of the starred arrangements, 0.19230, gives the probability for getting exactly three genes on one chromosome and no more than two genes on any other.

I am indebted to Dr. C. A. Lamb for first drawing my attention to this problem. I wish to express my gratitude to Dr. Madge T. Macklin, Mrs. Caroline R. Madison, and Dr. E. L. Green for many helpful discussions and suggestions.

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NATURAL SELECTION AND EGG SIZE IN POULTRY

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It has long been recognized that maximum hatchability in chickens and turkeys is obtained with eggs of intermediate size (Landauer, 1948). The simplest interpretation of this phenomenon is that an absolute physiological optimum size of egg exists for a given set of incubation conditions, higher embryonic mortality being found in eggs that are larger or smaller than this optimum. It now appears that this explanation somewhat oversimplifies the situation, and that the relationship between egg size and hatchability (or more properly reproductive fitness) involves an interaction between artificial and natural selection pressures or the phenomenon designated as genetic homeostasis.

This term suggested by Lerner (1950) refers to the tendency of a population to maintain its genetic composition at an adaptive peak in the face of artificial selection pressures for one or more traits, and seems to be related to what Darlington and Mather (1949) have termed genetic inertia. The self-equilibrating tendency of a population has been demonstrated in a selection experiment with poultry reported by Lerner and Dempster (1951). A line of chickens was subjected to selection for increased shank length. In the early generations genetic gains commensurate with those expected on the basis of an additive gene-action hypothesis were obtained. After a period of such successful selection, progress ceased, in spite of the fact that there was no loss in genetic variability of the character selected for. It was determined that at least one of the reasons for the plateau was traceable to the pressure of natural selection counteracting that of artificial selection. In the plateau period, the fitness (relative number of offspring produced) of extreme deviates (that is, birds with the longest shanks) was below that of the individuals closer to the mean of the population. As a result the population remained at a stationary level, representing an equilibrium between artificial selection for long shanks, and natural selection for fitness.

The situation observed in this instance parallels closely that in the Drosophila selection experiments of Mather and Harrison (1949). The similarity is accentuated by the fact that in the generation following the last one reported by Lerner and Dempster advance in shank length was resumed, suggesting that a new adaptive peak permitting higher levels of this trait is in process of being established by an adjustment of the genetic composition of the population to the conditions imposed on it by the particular selection pressures applied.

One may expect that a balance of forces will operate in a similar manner with respect to any specific quantitative trait, but a precise demonstration of such a balance may be rather difficult to obtain because of various complicating factors. The case of egg size in birds, it appears upon investigation, offers an opportunity for just such a demonstration. On the basis of the data presented by Lerner and Gunns (1952), auxiliary information from the experimental flock they used in their study, and of an examination of the relevant data in the literature, it can be shown that natural selection does affect egg size in flocks of domestic birds. The direction of this effect is, as expected on the basis of the considerations noted above, counter to the direction of artificial selection, the mean egg size in a population representing the interaction of these two forces.

EXPERIMENTAL EVIDENCE

The experiment conducted by Lerner and Gunns consisted of incubating eggs from a sample of 189 birds, representative with respect to the mean and range of egg weight of a generation of a flock of chickens selected primarily for egg number. The parent flock in the course of its 18-year history has been subjected to mild artificial selection for egg size. Attempts were made to maintain an average spring egg weight in the neighborhood of 56-57 grams. As may be seen from the data given by Lerner and Dempster (1951), the mean egg weights of birds selected as parents in different generations fluctuated about this level. When egg size dropped, the pressure for large eggs was increased; when egg size came up to the desired level, this pressure was relaxed.

In the experiment all birds in the sample were given an equal opportunity to produce chicks in the course of a series of hatches extending from December to June. All eggs laid on 70 days (scattered in this period) by all of the birds concerned were incubated, and records of the actual number of chicks produced by each were taken. Three statistics were thus available for each individual: number of eggs laid, number of chicks hatched, and the percentage of hatchability.

The birds were then grouped according to the characteristic weight of eggs they produced into arrays centering around the mean of the whole population. It was found that the maximum values for all three measurements considered fell into the intermediate egg weight classes. By fitting second degree polynomials to these data, points of maximum fitness (as measured by the three criteria used) with respect to egg weight could be established. All points were found to be below the mean egg weight of the population. It should be noted here that spring-hatched birds in the first laying year (such as were studied here) show increases in their egg size from beginning of lay in the fall to the next spring. In the experiment described it mattered little whether the birds were characterized for egg weight in the fall or in the spring. In either case the optimum egg weight for fitness was below the appropriate mean.

The experiment discussed then shows that the pressure of natural selection in this population is in the negative direction from the mean, artificial selection maintaining average egg weight somewhat above the optimum for fitness. Hence one might expect that, should the population on which this study was carried out be permitted to reproduce itself without any artificial selection, it would presumably reach an equilibrium mean egg weight, representing an adaptive peak not far removed from the optimum value computed (discrepancies between optima as computed and the equilibria may arise should differential post-hatching mortality exist). No direct evidence of this type is as yet available for the present population, but an approximation of the equilibrium point can be made from the data furnished by Mr. F. T. Shultz, who is presently working with inbred lines derived from the flock under study.

The general outline of this inbreeding study has been given by Lerner (1950). The information pertinent to the present problem comes from the behavior of four lines, in which selection for egg weight was suspended, and of the crosses between them. The lines were isolated from the parent flock in 1945, the 1950 population contemporary with the birds used in the egg weight study representing the sixth inbred generation. At the beginning of the experiment two of the lines were selected for low and two for high November egg production. In the later years because of inbreeding degeneration affecting reproductive fitness the degree of selection practiced was very low if at all existent. Though it is clear that selection for egg number is bound to have some effect on egg size (see, for example, Jull, 1930, on the relation between these two properties), it may be expected that on the average, selection being in both directions, the equilibrium point may be estimated from the means of the lines. The case is strengthened by the addition of information available from reciprocal crosses between the two

TABLE 1

AVERAGE EGG WEIGHTS IN 1950 INBRED LINES AND CROSSES

Line or cross	November		April	
	Number of birds	Mean egg weight	Number of birds	Mean egg weight
Low Number 1	35	47.00	24	55.45
Low Number 2	34	45.88	34	52.91
All low	69	46.45	58	53.96
High Number 1	13	43.31	9	47.51
High Number 2	8	46.50	7	52.27
All high	21	44.52	16	49.59
All inbred lines	90	46.00	74	53.02
Crosses between low number lines.	18	48.00	22	55.19
Crosses between high number lines	39	47.64	33	52.68
All Crosses	57	47.75	55	53.68
All birds	147	46.48	129	53.30
Computed optimum: Unweighted		46.29		53.21
Weighted for number of birds in each pen studied		46.30		53.29

low number lines and between the two high number lines. Table 1 shows the mean egg weights of the different lines and crosses.

It may be seen that while some variation between lines exists it is relatively small, particularly for November. It may also be seen from the last three lines of the table that the computed optima are remarkably close to the estimates of equilibrium points. Since the mean egg weights of the birds in the study previously described were higher (48.09 grams for November and 56.39 grams for April), it appears that our interpretation of the phenomenon observed is well supported by this additional evidence.

EVIDENCE FROM THE LITERATURE

Further, confirmation may be sought in the literature on the relation of hatchability to egg size. Since in most flocks of domestic poultry some selection for larger egg size is practiced, it may be expected that the situation described above will prevail in other cases, that is, that the optimum egg weight for reproduction will fall below the mean.

Unfortunately, despite the voluminous data published on the relation between egg weight and hatchability, there are only a few reports which can be used for our analysis, the majority containing material on samples of eggs biassed in different ways. Either the birds from which the eggs were incubated or the eggs themselves were selected in a fashion which would interfere with the determination of the mean and the optimum egg weights.[1] For instance, Hébert and Laugier (1943) set equal numbers of eggs in different weight classes; or as it occurred in one of the sets of data presented by Scott and Warren (1941), the frequency distribution was such that the lowest egg weight class had the largest number of birds, rather than being of the quasi-normal type expected (and usually realized) in unselected samples.

Other reasons for rejection of data for the purposes of our analysis include the presentation of the material in a form combining the results from several years, and even more objectionable from our standpoint, from different seasons. In such instances (for example, Axelsson, 1932) the mean egg weight of the population for a given time of the year is impossible to determine. Still other sets of data in the literature available to us show irregular distributions of hatchability percentages (Benjamin, 1920), so that points of maximum fitness cannot be determined. Finally, others (three of the five sets of Funk, 1934; Warren, 1934; Shibata and Murata, 1936; the second set of Scott and Warren, 1941) show maximum hatchability for the lowest egg weight arrays. It is tempting to speculate that in such cases (all data being from breeding flocks) artificial selection for large egg size was more intense than in the other material under consideration. This, however, would be an obvious rationalization, and it is more proper to set these data aside as not demonstrating the more commonly observed curvilinear relation between hatchability and egg weight.

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[1] That selection of birds from which eggs are set can seriously affect the expression of the phenomenon under discussion is illustrated by the fact that Lerner and Dempster (1951) in dealing only with birds selected for breeding from the same flock as studied by Lerner and Gunns (1952) failed to find any obvious relationship between egg weight and fitness.

All the other sets of data located have been analyzed for mean weights and points of maximum hatchability. It should be noted that the analysis of these can be considered to be valid only as an approximation: (1) the

TABLE 2

RELATION BETWEEN OBSERVED MEAN EGG WEIGHT AND COMPUTED EGG WEIGHT, OPTIMUM FOR HIGH HATCHABILITY

Set no.	Population studied	All eggs set		Fertile eggs set		Reference‡
		Mean egg wt.	Optimum egg wt.	Mean egg wt.	Optimum egg wt.	
1.	W. Leghorn pullets	55.34	53.66			Dunn, 1922
2.	" " "			55.41	53.04	" "
3.	W. Leghorn hens	57.77	52.33			" "
4.	" " "			57.69	48.69	" "
5.	Chickens, breed not specified	56.11	57.54			Halbersleben and Mussehl, 1922
6.	Chickens, breed not specified			56.01	54.85	Halbersleben and Mussehl, 1922
7.	B. P. Rock pullets	55.46	54.44			Jull and Haynes, 1925
8.	" " " "			55.47	52.79	Jull and Haynes, 1925
9.*	R. I. Red pullets			59.65	57.77	Funk, 1934
10.*	R. I. Red hens			60.85	55.95	Funk, 1934
11.	Turkeys, mixed breeds			77.33	79.94	Byerly and Marsden, 1938
12.	Turkeys, breed not specified			88.26	63.14	Insko, MacLaury and Baute, 1943
13.	Turkeys, breed not specified			89.62	79.39	Insko, MacLaury and Baute, 1943
14.	Turkeys, breed not specified			86.87	89.28	Insko, MacLaury and Baute, 1943
15.	Turkeys, breed not specified			89.00	77.80	Insko, MacLaury and Baute, 1943
16.	Turkeys, breed not specified			87.63	83.20	Insko, MacLaury and Baute, 1943
17.*	Leghorn pullets (November)	48.64	47.44			Lerner and Gunns, 1952
18.*	Leghorn pullets (April)	57.03	51.93			Lerner and Gunns, 1952
19.*†	Leghorn pullets (November)	48.64	46.30			Lerner and Gunns, 1952
20.*†	Leghorn pullets (April)	57.03	53.29			Lerner and Gunns, 1952

* Bird averages rather than individual eggs.

† Based on number of chicks produced rather than on percentage hatch.

‡ Addendum: The paper by Skoglund, Tomhave and Mumford (1948, Poultry Sci. 27:709-712) was inadvertently overlooked when Table 2 was being compiled. Their extensive data appear to substantiate the thesis advanced here, particularly in the demonstration of concurrent increases in the mean and optimum egg weights with advancing age of the birds.

means computed are for populations of eggs set, and not for the parental flocks; (2) information on the type of sampling is generally lacking; (3) data on hatchability only, rather than on reproductive fitness are available.

Nevertheless, as it may be seen from table 2 in all but three sets of data (appearing in italics) the optimum egg weight as computed under the direction of Dorothy C. Lowry in the same manner as for the data of Lerner and Gunns, falls below the mean. No immediate explanation for the departure from expectation of two of these (5 and 14) is available. The third one (11), as it happens, provides a striking confirmation of the thesis advanced, since it is derived from a turkey flock, in which selection has been practiced for small body size, and hence for small egg size. In such a situation the prediction from our hypothesis is that the optimum will be higher than the mean, as is clearly the case. Thus in 18 out of 20 available examples (not all of which are, of course, independent of each other) the expectation is fulfilled.

There seems to be little doubt that natural selection is a force of appreciable magnitude in the determination of the average egg size exhibited by domestic populations of birds. The differential reproductive fitness of birds with different genotypes for egg size may be due to a reduction from the potential number of offspring in any one of the stages where losses can occur. Thus the number of eggs produced by each dam, their fertility and their hatchability may all be involved in this process. It is quite likely that in different cases one or more of these operate. Thus in the experiment of Lerner and Gunns (1952) both egg production and the hatchability of all eggs set showed a curvilinear relation to egg weight. In the material of Kumanov (1948), which was not included in our table 2 because of the uncertainty regarding the nature of the data (it appears as if pooling of seasons and years, such as made by Axelsson, 1932, was also resorted to here), there is a suggestion that fertility may be implicated, as it may be seen from the following figures:

	Mean egg weight	Optimum egg weight	
All eggs	56.24	52.41	(based on fertility)
		57.78	(based on hatchability)
Fertile eggs	56.14	54.01	(based on hatchability)

On the other hand, the data of Düzgüneş (1950) on the reproductive fitness of inbred lines indicate that the hatchability of fertile eggs may be the major contributor to variance in fitness. The precise physiological mechanism involved in any given instance of genetic homeostasis is thus a subject for future investigation.

Similarly, the particular genetic mechanisms underlying the balance between natural and artificial selection which determines the observed average egg size in each case need thorough study. Simple pleiotropy or linkage are possible; but overdominance, epistasis, and balanced heterozygosity of chromosome segments or whole chromosomes may also be involved. Examples for most of these mechanisms can be adduced in different situations (Caspari, 1951; Nybom, 1950; Mather and Harrison, 1949; Dobzhansky, 1947). Which ones are specifically applicable to the situation at hand cannot be said at this time. It is clear, however, that in animal breeding practice, the phenomenon of genetic homeostasis must be taken into account in the formulation of efficient breeding systems.

SUMMARY

Experimental evidence is given for the operation of natural selection for egg size in populations of domestic fowl. As a rule the counter-force of artificial selection in commercial flocks maintains average egg size above the optimum for reproduction.

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SPERMATOGENESIS OF THE HORNED LIZARD PHRYNOSOMA CORNUTUM[1]

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INTRODUCTION

One of the important cytological aspects of vertebrate phylogeny deals with the investigation of the mutual connections between the chromosomes of reptiles and those of birds and mammals. Results obtained from the study of birds have shown the male homozygous and the female heterozygous for sex, whereas in mammals the opposite condition prevails. The biological basis for this difference is not known, but it is possible that a systematic investigation of reptilian chromosomes may yield information of value in explaining this situation. Investigators in several parts of the world have worked on this problem and among them are: Matthey (1949) in France; Nakamura (1935), Makino (1949), (1951), and Momma (1949) in Japan; as well as the earlier work of Painter (1921) in this country. Results from chromosome studies on 85 species of reptiles have been reported according to Makino and Momma (1949). In most of these studies, the emphasis has been placed upon the determination of the diploid number of chromosomes as observed in the spermatogonia.

The purpose of this investigation is to follow the chromosomes through spermatogenesis of a reptile, the horned lizard, *Phrynosoma cornutum* (Harlan).

MATERIALS AND METHODS

The specimens used in this study were collected during their breeding season from May through August, 1950, in the vicinity of Lubbock and Kingsville, Texas. The animals were killed by decapitation following the recommendation of Painter (1934) who has suggested the use of anaesthetics tends to affect the chromosomes. The testes were removed, placed in fixing fluid, and the tubules teased apart to facilitate penetration by the fixative.

Three methods of fixation were used in this investigation. These included: (1) Bouin's (1936), modified by increasing the acetic acid content from 5 to 20 cc.; (2) Painter's modification of Bouin-Allen's fluid (1934); and (3) an unpublished fixative developed by Cross (1950) consisting of 60 cc saturated picric acid solution, 25 cc of 37 percent formaldehyde, and 25 cc glacial acetic acid. Prior to using, 1.5 grams of picric acid and 3 grams of urea were added. Following fixation the tissues were dehydrated in the usual series of alcohols, cleared in xylol, and embedded in paraffin.

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[1] A thesis submitted to the Graduate Faculty of Texas Technological College in partial fulfillment of the requirements for the degree of Master of Arts. The study was under the direction of Dr. J. C. Cross.

The increase in the acetic acid content of Bouin's fluid facilitated the spreading of the chromatin material so that the morphological elements of the nucleus could more easily be examined in detail. This method was also an improvement in fixation of primary and secondary spermatocytes. However, the spermatogonia fixed in this manner were not suitable for counting the chromosomes of the diploid complex, but the prophase stages were satisfactory. Painter's modification of Bouin-Allen's (1934) was found to be unsatisfactory in this work. However Painter (1921), working on the spermatogenesis of reptiles, used this method of fixation with excellent results.

In this investigation the fixative developed by Cross (1950) was the most successful of those tried because excellent conditions were obtained for the study of spermatogonia. The chromosomes were well spread with a minimum amount of overlapping and could be counted in most of the complexes. The prophase stages, as well as the primary and secondary spermatocytes, were also good for this purpose.

The staining technique was a 3 percent ferric ammonium sulfate mordant followed by four hours in 0.5 percent hematoxylin. Destaining was carried out in 1.5 percent ferric ammonium sulfate followed by ten minutes in saturated picric acid. The picric acid dissolved some of the cytoplasmic inclusions and permitted critical observation of the chromosomes. No counterstaining was employed in this study.

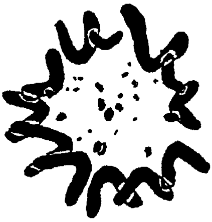
RESULTS

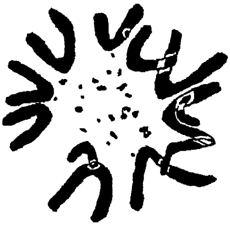
Spermatogonia. Dividing spermatogonia (figs. 1 and 2), when seen from the equatorial plate, polar view, showed an outer circle of large metacentric or V-shaped chromosomes surrounding the dot-like ones which were located in the center of the cell. Throughout this study the V-shaped elements are referred to as macro-chromosomes whereas the dot-like ones are termed micro-chromosomes. Twelve macro-chromosomes and 24 micro-chromosomes were found in the spermatogonia of *Phrynosoma cornutum.* Therefore, the diploid number is 36. The shape of the macro-chromosomes was so distinctive that it was not difficult to pair the homologous mates, as shown in fig. 3. However, no attempt was made to pair the micro-chromosomes due to their small size and irregular appearance. No chromosomal satellites were observed, and the staining technique obscured the centromere as well as the nucleolar organizer.

In general, most of the small chromosomes were well distributed and centrally located within the metaphase plate, but some were very close to the peripherally located larger elements, therefore making their enumeration difficult. Twenty-four micro-chromosomes are seen in both of the spermatogonia shown in figs. 1 and 2. This number corresponds with the 12 micro-chromosomes found in the primary spermatocyte.

Primary Spermatocyte. The leptotene stage of the prophase, as illustrated in fig. 4, presented a fine network of chromatin material with very

PLATE I





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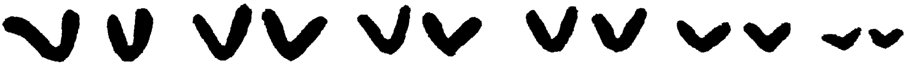

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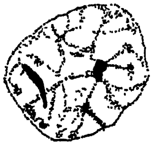

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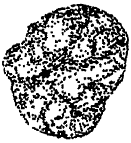

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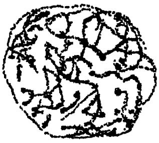

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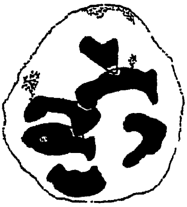

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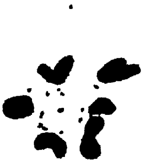

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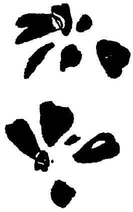

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long and slender filaments. At this stage the chromomeres were visible as small beads extending the entire length of the chromosomes. Two large nucleoli are shown in fig. 4, and there was no evidence of the micro-chromosomes at this stage. No zygotene stages were found.

The amphitene stages of the prophase, as shown in fig. 5, were very numerous. In general, the chromosomes exhibited a lack of uniformity, and as a result the cell had a rough appearance. The nucleoli, which were visible in the leptotene cells, were not observed in succeeding stages.

In the early pachytene, as illustrated in fig. 6, the chromosomes became shorter and had a rather coarse appearance, but this was possibly due to the contraction of the chromatin matter. No polarization of the chromosomes was visible in any of the observed earlier stages.

Diakinesis, as shown in fig. 7, exhibited six large tetrads and was characterized by a constriction of the filaments to such a degree that they became large broad bands of chromatin. This constriction was to a much greater degree than that found in any of the earlier prophase stages. The stippled areas, as indicated in fig. 7, represent the regions in which granulated chromatin material was visible.

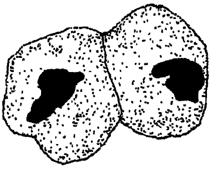
The polar view of the primary spermatocyte, illustrated in fig. 8, indicated that the haploid number of chromosomes was 18. This stage contained 6 macro-chromosomes and 12 micro-chromosomes. The latter elements were difficult to locate, as there was always the possibility that some of them might be obscured by the macro-chromosomes.

Many of the primary spermatocytes showed the tetrads in the metaphase side-view. There were 6 of the larger chromosomes present, most of them being hat-shaped; but due to their large size there was a great deal of overlapping. In general, the micro-chromosomes were not visible in most of the side views of the metaphase plates of the primary spermatocytes. In a few cells one or two of the micro-chromosomes were observed. However, in the majority studied, they were covered by the larger tetrads.

In fig. 9 a primary spermatocyte is represented in the anaphase stage as its chromosomes are grouping to form the secondary spermatocyte. This figure shows both sets of dyads as they were migrating towards their respective poles. The secondary spermatocytes had six large chromosomes. It was not possible to make an accurate count of the micro-chromosomes of the secondary spermatocytes.

Metamorphosis of Sperm. The metamorphosis of the sperm of *Phrynosoma cornutum* could be easily traced by the staining method used in this investigation. After the chromosomes had reached their respective poles at the conclusion of telophase II, they lost their identity and massed together. Following this, the cells separated, forming the two spermatids as shown in fig. 10. At this stage of development a general grouping of the chromatin material tookplace and a small sphere was formed within the cell membrane as illustrated in fig. 11. Elongation of the nucleus took place at this time and continued until this structure assumed a rod-shaped appearance and was located in the center of the cell as shown in figs. 12 and 13. At this

PLATE II



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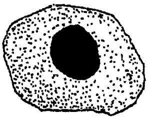

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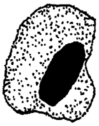

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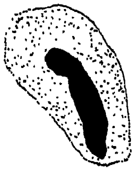

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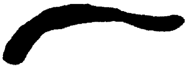

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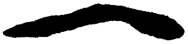

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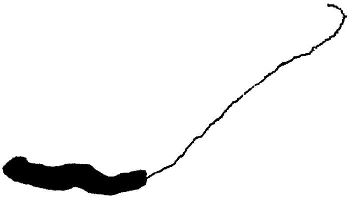

16

time the chromatin began to migrate towards the edge of the cell membrane and soon was free of the cytosome (figs. 14 and 15). After this stage, the whip-like flagellum was formed. With the staining methods employed in this study, it was not possible to trace the formation of the acrosome, neck, or middle piece.

DISCUSSION

The spermatogenesis of *Phrynosoma cornutum* is typical of those previously described for the family Iguanidae (Painter, 1921), but two important differences exist: (1) the formation of sex chromosomes and (2) the absence of giant spermatozoa. Painter (1921) published plates illustrating the passage of the undivided X-chromosome to one pole of the first division spindle in animals representing the genera *Anolis* and *Sceloperus*. However, this early migration of one chromosome to the pole was not observed in this study of *P. cornutum.*

It was not possible to accurately distinguish the sex chromosomes from the autosomes in this species. Due to the pairing of the macro-chromosomes, the X-Y condition was not apparent in these elements. This difference may be genetic, but the morphology of the chromosomes offered no explanation. However, Nakamura (1935), working near Kyoto, Japan, found evidence for sex chromosomes in male lizards belonging to the genus *Takydromus.* The spermatogonia in the three species studied revealed 38 chromosomes. Thirty-six of these were of various sizes and therefore this investigator was unable to sort them into categories of macro- and micro-chromosomes. He concluded that the other two chromosomes were the sex chromosomes representing the X-X condition. It is possible that the X-X or X-Y arrangement may exist in the micro-chromosomes of the horned lizard, but due to their small size, it was not possible to pair these elements. If sex is determined by the macro-chromosomes, then due to their perfect pairing the X-X condition would likely prevail in *P. cornutum.*

The horned lizard, which belongs to one of the families studied by Painter (1921), fits into the typical chromosomal pattern of reptiles as described by him. He pointed out that the chromosomes of lizards show a sharp division into size groups of macro- and micro-chromosomes. In all the lizards examined, 12 V- or U-shaped macro-chromosomes were present, but variation existed in the numbers of the smaller elements.

SUMMARY

A study has been made of the spermatogenesis of the Texas horned lizard, *Phrynosoma cornutum* (Harlan). Dividing spermatogonia showed 36 chromosomes; 12 of these were V-shaped macro-chromosomes and 24 were dot-like micro-chromosomes. In the primary spermatocytes there were six macro-chromosomes and twelve micro-chromosomes. Some of these stages, when seen in metaphase side view, had six hat-like tetrads. In polar view, the secondary spermatocyte presented six large chromosomes arranged in a circle surrounding the micro-chromosomes. The metamorphosis of sperm is described. No evidence was found to suggest the presence of sex chromosomes in this species. However, the X-X condition may exist in the macrochromosomes.

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EXPLANATION OF FIGURES

All the drawings were made with the aid of an Abbe camera lucida and at a magnification of X3000.

Plate I.

FIGURES 1 and 2. Dividing spermatogonia of *Phrynosoma cornutum*, as seen from the equatorial plate, polar view, showing thirty-six chromosomes.

FIGURE 3. Serial alignment of macro-chromosomes of fig. 1.

FIGURE 4. Leptotene stage showing a fine network of chromatin.

FIGURE 5. Amphitene stage.

FIGURE 6. Pachytene stage.

FIGURE 7. Diakinesis stage.

FIGURE 8. Polar view of the primary spermatocyte showing eighteen chromosomes.

FIGURE 9. Anaphase stage of the primary spermatocyte as its chromosomes are grouping to form the secondary spermatocyte.

Plate II.

FIGURE 10. Spermatids.

FIGURE 11. A single spermatid.

FIGURE 12. Elongation of the chromatin material.

FIGURE 13. Migration of chromatin towards the edge of the cell membrane.

FIGURES 14 and 15. Chromatin free from the cytosome.

FIGURE 16. Spermatozoon.

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THE FATE OF YEAST IN THE DIGESTIVE TRACT OF DROSOPHILA

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The importance of yeasts as a food for Drosophila was well established by Delcourt and Guyenot (1910), Guyenot (1913), and Northrop (1916 and 1917). In line with this, it has been customary for many years for biologists, and particularly geneticists, experimenting with Drosophila to use yeast in the food of their fly cultures. Considerable information on the nutritive value of yeasts to Drosophila is available in reports by Baumberger (1919), Chevais (1942), Chiang and Hodson (1950), Gordon and Sang (1941), Robertson and Sang (1944), and others. There is, however, no information on the fate of yeasts ingested by Drosophila or the rate at which they are digested. Recently the authors were concerned with the isolation of yeasts from the intestinal tracts of Drosophila flies collected in the mountains of central and southern California. The flies were shipped to Berkeley and yeast isolation made in the shortest time possible after collection. The initial collections of flies yielded relatively few yeasts. Since the crops of the flies were well distended at the time of collection, it was presumed that the yeasts were digested before isolations were attempted in the laboratory. It became necessary to develop a procedure for the transportation of flies to the laboratory with a minimum destruction of yeast, or abandon the project. In the development of this procedure, certain data of interest to Drosophila workers were obtained. These are reported below:

DIGESTION OF YEAST CELLS BY DROSOPHILA

D. pseudoobscura flies were held in a bottle at room temperature without food for twenty-four houts before the introduction of a paste of bakers' yeast on a glass slide. The flies were permitted to feed on the yeast paste for two hours, after which time they were well fed, as indicated by distention of the entire abdomen. The flies were then removed and placed in clean bottles which were stored at different temperatures. At various intervals, flies were withdrawn from each bottle and the alimentary tract removed from plating. The number of yeast cells in the alimentary canal of each fly was determined by the quantitative plating method as described by Henrici and Ordal (1948). The results of these tests are given in table 1.

It is obvious that digestion is very rapid at room temperature and much slower when the temperature is appreciably lower. The crops of flies held at room temperature were almost empty 24 hours after feeding. This is in agreement with the findings of Dobzhansky and Epling (1944) that contents of the crop pass into the gut in about 24 hours after ingestion of the food. On the other hand, crops of flies stored at 0°C. appeared full at the time of dissection, but the extent of distention decreased when the storage period was increased. The experiment showed the importance of cooling flies as soon as they are collected, so the internal flora can be studied without much change during transportation. Accordingly the writers adopted the following procedure for the handling and transportation of flies from their native habitat to the laboratory.

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TABLE I

SURVIVAL OF YEASTS IN D. PSEUDOOBSCURA STORED FOR VARIOUS PERIODS OF TIME AT DIFFERENT TEMPERATURES

Storage time after feeding	Average number of cells per alimentary tract			
	Room temperature	15° C	5° C	0° C
Immediately after feeding	150,000	150,000	150,000	150,000
24 hours	65	300	3,200	93,000
48 hours	0	160	1,500	62,000

The flies were collected in a test tube which was cooled immediately by placing in a "super-ice" package. This is a commercial package consisting of an insulated box containing a frozen block[3] which holds the surrounding temperature at about 0°C. The container with flies was shipped on the evening of the collection enabling the isolation of yeasts from the flies early the next morning.

FATE OF YEAST CELLS IN THE DIGESTIVE TRACT OF DROSOPHILA

Although many investigators have shown the importance of yeast in the nutrition of Drosophila, there is no information indicating the effect of the digestive mechanism of Drosophila on yeast cells. In our work, the question arose as to whether or not fly pellets might be used as a source of cultures as indicated by the work of Hedrick and Burke (1950).

Flies (*Drosophila pseudoobscura*) were permitted to feed on bakers' yeast as indicated above. Fecal pellets were then collected on slides for examination. The direct microscopic examination of fecal pellets showed that they were composed almost entirely of intact rather than broken yeast cells. There was no indication of cell wall breakage during the digestive process. Upon applying a solution of methylene blue (1:100,000) to fecal pellets, the yeast cells stained instantly, indicating that they were killed by passage through the digestive tracts. Furthermore, microscopic examination revealed that each pellet contained 20-30 yeast cells and that practically all the cells were more or less empty. There was no evidence to indicate that more than a stray yeast cell, if any at all, could survive passage through the intestinal tract of *D. pseudoobscura.*

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[3] This block is a commercial preparation consisting of a mixture of sawdust and certain chemicals enclosed in a coarse fiber paper covering. In using the block, it is first submerged in water, then frozen at −6°F or less. When placed in the insulated box, it remains cold for a much longer period than does ice.

According to Lamanna and Mallette (1950), yeast cells are gram positive because of the presence of ribonucleate. Therefore, if this substance is removed, the cells should become gram negative. This proved to be the case upon application of the gram stain to yeast cells in fecal pellets, indicating the removal of ribonucleate during passage of the yeast cells through the digestive tract.

ACKNOWLEDGEMENT

The authors are indebted to Professor M. A. Joslyn for his many helpful suggestions.

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SELECTIVE DIFFERENCES BETWEEN MALES AND FEMALES IN DROSOPHILA ROBUSTA

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INTRODUCTION

Carson and Stalker (1948) discovered that females of *Drosophila robusta* Sturtevant collected in the late summer and autumn near St. Louis, Missouri, were almost always virgin or uninseminated. A similar phenomenon was noted in collections of this species at Englewood Cliffs, New Jersey (Levitan, 1951a). Chromosomal analysis of the diapausing females of this species thus yielded an estimate of the frequencies of the gene arrangements in adult females of the population ("adult female sample"), in addition to the adult male and egg samples available as in other species. This made possible a comparison of the frequencies of the gene arrangements in the two sexes. The data suggested significant differences between the sexes for several arrangements, but the results could not be checked by further fall collections in that locality. Collections of *D. robusta* were, however, resumed near Blacksburg, Virginia. In this population selective differences between male and female carriers of the same gene arrangements have also been observed.

MATERIALS AND METHODS

Collections were made in North Crumpacker Woods, about one mile from the campus of Virginia Polytechnic Institute, in Montgomery County, approximately 2,100 feet above sea level. The vegetation is of the oak-hickory association, including also abundant wild cherry, black locust, dogwood, black haw, poison ivy, and blackberry. The methods of collection and analysis were described in a previous paper (Levitan, 1951a). To increase the accuracy of the analysis of adult samples, the salivary gland chromosomes of eight larvae were studied in the offspring of each male or virgin female collected. Determination of the chromosomal constitutions of females inseminated in nature was possible only when the single offspring (larva) examined was homozygous for a chromosomal arm; this gave evidence of one arrangement of that arm carried by the collected fly.

RESULTS

Tables 1–3 show the results obtained in the spring and fall of 1950. The spring data are from two samples (April 24–May 13 and May 30–June 8) and the fall data from three samples (August 24–September 4, September 23–October 1, and October 23–November 8). Within each season the samples are not significantly heterogeneous. In the adult female samples, 24 of the

TABLE 1

FREQUENCIES (IN PER CENT) OF X-CHROMOSOME ARRANGEMENTS IN NORTH CRUMPACKER WOODS, BLACKSBURG, VIRGINIA, 1950

Sample	n	XL	XL-1	XL-2	n	XR	XR-2
A. Adult females							
Spring	48	43.8	56.2	0.0	61	24.6	75.4
Fall	251	45.4	45.8	8.8	252	20.2	79.8
B. Adult males							
Spring	70	28.6	64.3	7.1	70	18.6	81.4
Fall	152	44.1	42.1	13.8	152	21.0	79.0
C. Totals*							
Spring	206	36.4	56.8	6.8	206	25.2	74.8
Fall	411	45.0	44.3	10.7	411	20.7	79.3
Grand total	617	42.1	48.5	9.4	617	22.2	77.8

*Differences between these totals and the sum of the adult samples is accounted for by the inclusion of all the egg sample data in the totals.

chromosomes in the spring and 246 of the chromosomes in the fall for each arm are from analyses of virgin females.

The frequencies of the arrangements in the left arm of the X-chromosome and in the left arm of the second chromosome show highly significant differences between spring and fall in the total data. Data from the spring, 1951, collections indicate that the changes are probably cyclic (Levitan, 1951b). The situation is presumably analogous to that observed in *D. pseudoobscura* by Dobzhansky (1943, 1948) and in *D. funebris* by Dubinin and Tiniakov (1946). However, males and females are not equally responsible for these changes. Thus, the differences between the spring and fall adult male samples for the XL arrangements are highly significant (Chi-square of 9.565 for two degrees of freedom), whereas the differences are not significant for the corresponding adult females. The significant changes in

TABLE 2

FREQUENCIES (IN PER CENT) OF SECOND-CHROMOSOME ARRANGEMENTS IN NORTH CRUMPACKER WOODS, BLACKSBURG, VIRGINIA, 1950

Sample	n	2L	2L-1	2L-2	2L-3	n	2R	2R-1
A. Adult females								
Spring	38	23.7	23.7	5.3	47.4	66	100.0	0.0
Fall	247*	25.5	19.8	19.4	34.8	252	94.0	6.0
B. Adult males								
Spring	140	25.0	22.1	11.4	41.4	140	89.3	10.7
Fall	303	28.7	22.8	21.8	26.7	303	88.4	11.6
C. Totals †								
Spring	276	26.1	22.5	11.6	39.9	276	90.2	9.8
Fall	567*	27.5	21.5	20.6	30.2	567	90.7	9.3
Grand total	843*	27.0	21.8	17.7	33.3	843	90.5	9.5

*Include one instance of arrangement 2L-5 (unpublished data), not included in the calculations discussed in the text.

†See footnote to Table 1.

the males occurred in the frequencies of XL and XL-1. On the other hand, the changes in the rarer arrangement, XL-2, are barely significant in the females (P about .04 for one degree of freedom) but not at all in the males. Similarly, the changes in the left limb of the second chromosome are highly significant in the males but not in the females. In females, significant changes are recorded only in 2L-2, since the test for heterogeneity between spring female 2L-2 and fall female 2L-2 give chi-square equal to 4.607, for which P is between .02 and .05 for one degree of freedom. In the males the changes are highly significant for both 2L-2 ($\chi^2 = 6.800$ for 1 d.f.) and 2L-3 ($\chi^2 = 9.601$ for 1 d.f.). The participation of both sexes in the 2L-2 changes accounts partly for the absence of a significant difference between the frequencies of this arrangement carried by the sexes in the fall; on the other hand, the considerable seasonal change in 2L-3 in males but not in females is reflected by the fact that the sexes do show some significant differences in the proportions of 2L-3 they carry ($\chi^2 = 4.480$, with P between .02 and .05 for 1 d.f.). In XL, males and females show their largest ostensible differences in spring, but these differences are not statistically significant. The frequency of XR among adult females appeared to fall, and XR-2 to rise, between spring and autumn with an opposite change in the males, but neither change proved to be statistically significant.

TABLE 3

FREQUENCIES (IN PER CENT) OF THIRD-CHROMOSOME, RIGHT ARM ARRANGEMENTS IN NORTH CRUMPACKER WOODS, BLACKSBURG, VIRGINIA, 1950

Sample	n	3R	3R-1
A. Adult females			
Spring	50	50.0	50.0
Fall	249	54.2	45.8
B. Adult males			
Spring	140	52.1	47.9
Fall	302	54.0	46.0
C. Totals*			
Spring	276	51.4	48.6
Fall	566	54.4	45.6
Grand total	842	53.4	46.6

*See footnote to Table 1.

Males seem to carry more 2R-1 and less 2R than females in both seasons. This difference is fairly significant in the fall data ($\chi^2 = 5.380$, $.02 < P < .05$ for 1 d.f.) and highly significant in the smaller spring data ($\chi^2 = 7.637$ for 1 d.f.).

Though 3R tends to be more common, and 3R-1 less common, in the fall than in the spring in all samples, the arrangements of the arm show no significant sexual or seasonal differences. Similar constancy was observed for 2L and 2L-1. In New Jersey 2L showed the most significant sex differences (Levitan, 1951a).

SUMMARY

Drosophila robusta collected in a Southwest Virginia woods show seasonal changes in gene arrangement frequencies in several chromosomal arms. Most of these changes are significant in males but not in females. The gene arrangements of one arm show no seasonal changes but differ significantly in the two sexes. The data indicate that the selection pressures on carriers of these gene arrangements are probably not identical in males and females.

ACKNOWLEDGEMENTS

The author is grateful to Professor A. B. Massey, of the Botany Section of the Department of Biology, Virginia Polytechnic Institute, for aid in identifying the vegetation of Crumpacker Woods, and to the Rockefeller Foundation for assistance in procuring the necessary equipment.

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THE SCENT-PRODUCING ORGAN OF THE MALE MONARCH BUTTERFLY

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Wherever the common milkweed grows in the east during the spring and summer months, we find, often in large numbers, the familiar orange and black milkweed butterfly, or Monarch. The entomologist, artist and color photographer have helped to make this one of the best known of the insects. Its interesting and amazing habits of migration have been recorded by writers and by the motion picture camera. One who has observed the emergence of the adult from the green, gold-studded chrysalis has had an experience never to be forgotten.

Although Monarch butterflies are figured in almost every book concerned with insect study, it is by rather careful examination of the butterflies themselves that the sexes are determined. The female may be distinguished by somewhat darker coloration due to rather wide bands of black scales along the wing veins. In the male, the veins are more narrowly margined with black, and there is present, on the upper surface of each hind wing, a small, black patch adhering closely, though not connected, to the second cubitus vein (fig. 1, above). Similar structures on the wings of male butterflies are quite common and constitute a universal type of scent-producing organ in these insects.

The scent organ of the Monarch butterfly is a slightly elevated black pouch about three millimeters in length and one and one half millimeters in width. The darkly colored scales which cover it are similar to those found in the dark areas of the wings (fig. 1, center). In freshly caught specimens these scales can be readily brushed off with a fine brush. For dried insects, on the other hand, scraping with a fine blade is necessary in order to remove the scales. Removal of the scales exposes a slightly rounded portion of the ectoderm, like a small pocket, somewhat darkened and covered with specialized cells and tiny pits. Breaking through the thin covering, the inner cavity of the sac can be studied. Here is a lining of tiny, black, overlapping scales. These can be examined by scraping the inner wall with a fine knife or flattened, sharp needle, and mounting them on a microscope slide. They are much smaller and more delicate than the surface scales and more heavily pigmented. These are the "scent scales," or "androconia." The latter is a term widely used by the entomologist, with the appropriate meaning "male dust." The inner wall of the sac itself is thin, yellowish, and with regularly arranged circular areas, so-called "scent cups." Each is covered with a thin cuticle bearing a minute central pore from which extends an extremely narrow, hair-like scale, similar to a seta. Fitted into such pores are the scale stalks or pedicels in contact

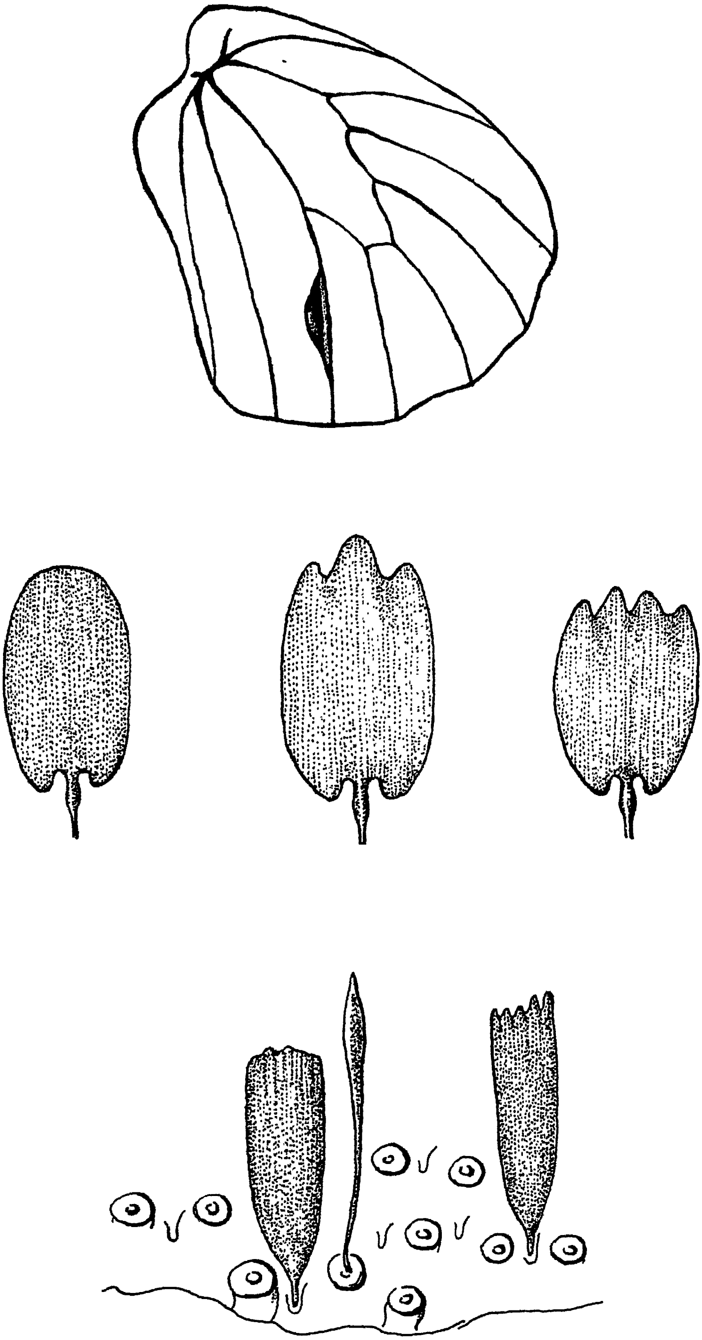

FIGURE 1. Above, hind wing of male showing scent organ; center, types of scales covering surface of scent organ; below, portion of inner wall of scent organ showing scent pits and scent scales.

with unicellular gland cells of the epidermis. In areas between the "cups" are pits in which are located the pedicels of wider scales (fig. 1, below). These two types of scales give off characteristic odors and would account for the term "scent scales" and for references to the sâc as a "scent gland" or an area of "sensory scales." Secretions from specialized gland cells escape in the regions of the pedicels to coat the surfaces of the scales. Thus, these modified insect "hairs" serve as outlets for cell secretions. The secretory substance has a faint, fragrant odor likened to the sweet milkweed blossoms with which the insect is associated. It may serve, in function, as a sex stimulant in that it is attractive to the opposite sex. In freshly captured specimens, the fragrance can often be detected over and above the more pungent and unpleasant general body odor. The latter, being decidedly repulsive, is considered a protection against enemies.

It would seem, therefore, that the scent organ is capable of producing a very effective perfume. Recognized by the relatively "poor" sense organs of man, to the butterflies it probably serves as a powerful stimulant readily distinguishable to members of the species. Being peculiar to the males, it would be an aid in finding their mates.

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BOOK REVIEWS

PALEONTOLOGY AND EVOLUTION

MARSTON BATES

The naturalist is interested in the diversity of living things: which means not only the description and analysis of the diversity of the present living world, but also the search for explanations. And this search for the explanation of living diversity, this preoccupation with organic evolution, provides the basic theme that binds all of natural history together and gives coherence to its superficially multifarious range of activities. Geneticists, paleontologists, ornithologists, taxonomists, mycologists, ecologists, morphologists, entomologists and so forth are all, after their distinctive fashions, attempting to throw light on the processes of evolution; even psychologists and physiologists, to the extent that they are studying the origin and diversification of their phenomena (the so-called "comparative" fields) are acting as naturalists. A new book on evolution must, then, be of interest to all of them.

And what a monumental book this is[1]: the summation of the thought and action of a long and fruitful life. Here Dr. Gregory has taken the essentials of his multitudinous observations and theories and fitted them neatly into the background of paleontological science, forming the whole into a unified scheme that can serve as a point of reference for what has been done, and as a point of departure for the observations of the future.

Essentially, this is a statement of the history of animals, especially of the vertebrates, as it is now known to us from the fossil record. It is, of course, illuminated by Dr. Gregory's extensive acquaintance with form and function in living animals; and the material is arranged so that from it there emerges a series of general principles—of anisomerism and polyisomerism, of divergence and convergence and progressive change. But it remains, still, an interpretation of history, an accumulation of observations and deductions based on an examination of the documents, the fossils.

A book like this stands in strong contrast to a book on evolution written by an experimental scientist, say a geneticist. Yet paleontology and genetics are equally sciences, and the methods of each are equally necessary to the development of an understanding of evolutionary processes. The contrast only serves to emphasize Conant's contention that it is folly to talk about "the scientific method": there are many sciences with many methods, all equally valid for their particular purposes; and the common denominator of science, whatever it may be, is surely not a method. The experimentalists sometimes seem to have got the upper hand, these days, in the peckingorder among scientists; and the ultra-centrifuge boys tend to be a little condescending toward the fellows with butterfly nets and geological hammers. But the observational scientists can stand on their record; there is no reason to give way at the food trough.

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[1] Gregory, William King. Evolution emerging; a survey of changing patterns from primeval life to man. New York: The Macmillan Co., 1951. Vol. I, xxvi + 736 pp. (text); Vol. II, vii + 1013 pp. (illustrations). $20.00.

Among the observational sciences, there is none with a more brilliant record than paleontology. Its accumulation of facts, deductions and generalizations is surely one of the great intellectual achievements of modern Western man, no less great because it is not susceptible to social perversion for the manufacture of bombs. It represents an extraordinary construction of interlocking concepts, based on the convergence of many lines of evidence, from bones and rocks and living organisms and patient examination of the action of physical forces. The paleontologists in our midst look like perfectly ordinary people, but they must represent some precious combination of genes and childhood influence that enables them to give the most painstaking attention to detail and yet keep their minds free for the most daring flights of imagination—flights that may be checked at any moment by some stubborn little fact, a molar tooth or fragment of a femur.

Yet paleontology, clearly, cannot tell us all about evolution. It gives us a frame into which our theories must fit, and provides us with ideas, with accounts of things that have happened and that must be explained in terms of processes that are now going on about us. The synthesis, the development of understanding of the total complex of processes, will perhaps become the function of some new sort of naturalist, who can combine the gleanings of the many special sciences: of the taxonomists, morphologists and comparative physiologists who have described the present diversity of living things; of the paleontologists who have accumulated the historical documentation; of the geneticists who have dissected the mechanisms of inheritance and variation; and of the ecologists who have formulated the principles of population dynamics and analyzed the operation of environmental forces. But before this new naturalist can begin to operate, each of us must formulate, synthesize and clarify the content of his special science, keeping in mind the needs and perspectives of this new, emerging field. This Dr. Gregory has done, providing us with a work that each of us can use in his special field, and that all of us can use in building toward this broader understanding of the living universe.

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The American Naturalist
635 W. 247 St.
New York 71, N. Y.

Ahlgren, Gilbert H., Glenn C. Klinghan, and Dale E. Wolf, 1951. Principles of Weed Control. 368 p., ill. $5.50. John Wiley and Sons, Inc., New York, N. Y.

Barclay-Smith, Phyllis, 1951. A Book of Ducks. 34 p., 16 colored plates by Peter Sheapheard. Penguin Books Limited, Harmondsworth, Middlesex, England.

Beaufort, L. F. de, 1951. Zoogeography of the Land and Inland Waters. VIII, 208 p., 10 figs. $5.00. Sidgwick and Jackson, London, England. (Distributed in U.S.A. by The Macmillan Co.).

This gives rather sketchy descriptions of the major zoogeographical regions, largely in vertebrate terms, with frequent reference to the paleontological record.

Brown, A. W. A., 1951. Insect Control by Chemicals. 817 p. $12.50. John Wiley and Sons, Inc., New York, N. Y.

Hegner, Robert W., and Karl A. Stiles, 1951. College Zoology. 6th Ed. 911 p. $6.00. The Macmillan Company, New York, N. Y.

Hesse, Richard, 1951. Ecological Animal Geography. 2nd Ed. Rewritten and Revised by W. C. Allee and Karl P. Schmidt. 715 p. $9.50. John Wiley and Sons, Inc., New York, N. Y.

Higman, Harry W., and Earl J. Larrison, 1951. Union Bay, the Life of a City Marsh. VIII, 315 p., ill. $4.00. University of Washington Press, Seattle, Wash.

A delightful book—"nature writing" at its best. It is an account in clean, unpretentious prose of wild events in a city marsh. It is mostly concerned with birds, but there is a lot about man and other mammals. No scientist could quarrel with the descriptions of behavior and ecological relationships, yet the narrative will surely pick up and hold the interest of a casual reader with no biological background.

Huntington, Ellsworth, 1951. Principles of Human Geography. 6th Ed. Revised by E. B. Shaw. XVIII, 805 p., many maps, photographs and diagrams. $6.25. John Wiley and Sons, New York, N. Y.

Shaw's revision includes the insertion of many warnings that climate and topography do not explain all human institutions, but the strong flavor of environmental determinism remains with hardly any intrusion of the concepts of contemporary anthropology. The book might more appropriately be called "the physical background of human geography."

Imms, A. D., 1951. Insect Natural History. XVIII, 317 p., 40 colored plates; 32 plates in black-and-white; 40 text figures; 8 maps. $5.00. The Blakiston Company, Philadelphia, Pa.

A volume of the British *New Naturalist* series. The enormous erudition of the author is expressed in a prose that is always clear and often amusing; the photographs, both in color and black-and-white, are masterpieces; even the diagrams are models of precision and clarity. In short, there is no better book about insect life for the general reader. It is about British insects, but these hardly differ from their American relatives. The book should make a splendid text. The instructor could translate the examples into local terms and the student would be left owning a beautiful book of enduring value.

Lapage, Geoffrey, 1951. Parasitic Animals. XXI, 351 p., 113 figs. $4.00. Cambridge University Press, New York, N. Y.

A good review of the types of parasite life histories and of adaptations of parasite and host. The author has packed an amazing amount of information between the covers of his book, though with more emphasis on physiology than on ecology. The material is too detailed, and the writing too pedestrian, for popular appeal, and the scholar will be disappointed by the complete absence of documentation; the book should have a useful place, however, as an introduction to the phenomena of parasitism for the student of general biology.

Lawrence, George H. M., 1951. Taxonomy of Vascular Plants. 823 p., ill. $7.95. The Macmillan Company, New York, N. Y.

Lederberg, Joshua (Ed.), 1951. Papers in Microbial Genetics. 303 p. $3.75. University of Wisconsin Press, Madison, Wis.

Pettingill, Olin Sewall, Jr., 1951. A Guide to Bird Finding East of the Mississippi. 659 p., ill. $4.00. Oxford University Press, New York, N. Y.

Pitelka, Frank A., 1951. Speciation and Ecologic Distribution in American Jays of the Genus Aphelocoma. 269 p. and 13 plates. $3.00. University of California Publications in Zoology, Vol. 50, No. 3. University of California Press, Berkeley, Calif.

Prescott, G. W., 1951. Algae of the Western Great Lakes Area. 946 p., 136 plates. $10.50. Cranbrook Institute of Science, Bulletin No. 31. Bloomfield Hills, Mich.

Pyrenson, Louis, 1951. Elements of Plant Protection. 538 p., ill. $4.96. John Wiley and Sons, Inc., New York, N. Y.

Rosendahl, Carl Otto, and Frederic C. Butters, 1951. A Guide to the Spring

Flowers of Minnesota. 8th Ed. 108 p., ill. $2.25. Burgess Publishing Co., Minneapolis, Minn.

Sanderson, Ivan T., 1951. How to Know the American Mammals. 161 p., ill. 35 cents. The New American Library of World Literature, Inc. (Mentor Books), New York, N. Y.

Science Reports, Fourth Series, Vol. XIX, No. 1, 1951. 132 p. Biological Institute, Tohoku University, Sendai, Japan.

Shull, A. Franklin, 1951. Evolution. 322 p. $5.00. McGraw-Hill Book Company, Inc., New York, N. Y.

Simpson, George Gaylord, 1951. Horses. 247 p. and 32 plates. $6.50. Oxford University Press, New York, N. Y.

Smith, Lyman B., 1951. Studies in the Bromeliaceae, XVI. 92 p. Contributions from the U. S. National Herbarium, Vol. 29, Part 10. Smithsonian Institution, Washington, D. C.

Stanford, E. E., 1951. Man and the Living World. 2d Ed. 863 p. $5.50. The Macmillan Company, New York, N. Y.

Transactions of the Kansai Entomological Society, 1950. Vol. XV, Part 2, Nov., 1950. 68 p. Kansai Entomological Society, Nishinomiya, Hyogo-Ken, Japan.

Westveld, R. H., and Ralph H. Peck, 1951. Forestry in Farm Management. 2d Ed. 340 p., ill. $5.00. John Wiley and Sons, Inc., New York, N. Y.

Williams, Jay, 1951. Fall of the Sparrow. 158 p. $3.00. Oxford University Press, New York, N. Y.

"Fall of the Sparrow" is essentially an obit for the many species of animals and plants which have lived on this planet, but which are no longer with us. Some of them became extinct many ages ago; some only yesterday; some have been extirpated by man either inevitably through the destruction of the only habitat in which they could survive, or through man's greed or ignorance or curiosity. Sometimes the name of the murderer of the last individual and the date of the crime is known; sometimes the species has just vanished like the Cheshire cat's smile, after the number of its individuals had decreased to the point at which it could no longer recover from such disasters as droughts, epidemics, forest fires, or severe winters. Climatic changes have been responsible for the disappearance of many species, but man is the greater villain particularly in recent times. The sprightly manner of the writing, close-knit and economical of words, points up the gloomy nature of the story, which is alleviated, however, by a cheerful chapter toward the end of the book. This gives a short account of the controls which have been set up in many countries as well as internationally to prevent indiscriminate destruction, and of the ecological studies which may be expected to slow down, if not entirely to stop, the extinction of still more species in the future.

Woodruff, Lorande L., and George A. Baitsell, 1951. Foundations of Biology. 7th Ed. 719 p. $5.50. The Macmillan Company, New York, N. Y.

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